ASSESSING SEASONAL TRENDS IN HARBOR SEAL (*PHOCA VITULINA*) DIET USING STABLE ISOTOPE ANALYSIS ALONG THE WHISKER

By

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A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of

Master of Science

in

Wildlife Biology and Conservation

University of Alaska Fairbanks

December 2017

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Abstract

Harbor seals (*Phoca vitulina*) that use tidewater glaciers in the summers to molt, pup, and breed have declined in recent years, which could be attributed to reduced prey availability caused by regime shifts in the coastal marine environment. Recording seasonal dietary trends of harbor seals in different years could improve our ability to test if regime shifts caused these declines. However, such analysis has not been possible, because wild harbor seals are difficult to recapture. Stable isotope analysis of serial sections of growing whiskers (mystacial vibrissae) can be used as a tool to assess diet over different seasons, but uncertainty about whisker growth status and shed dates have prevented accurate estimates of stable isotope deposition date in the past.

In Chapter 1, I characterized harbor seal whisker morphology to improve estimates of stable isotope deposition date. First, I measured 567 whiskers collected from wild harbor seals in the Gulf of Alaska from 2003 to 2012. Measurements included the length of a smooth root section (SRS), the length of the bumpy section, and the distance between each bump (inter-bump length; IBL). I found that the SRS was longer for spring-collected whiskers than fall-collected whiskers and matched the length of fully-grown, shed whiskers. These results suggest that the SRS can be used to differentiate whisker shed and growth status, and can be used to determine the sequence of whisker shedding by cohort in summer-captured seals. I also found that the mean IBL was correlated with whisker length and provides a proxy for whisker growth rate. I compared stable carbon isotope ratios along the three longest whiskers from 10 harbor seals and found that intra-individual patterns of whisker stable carbon isotope ratios became more synchronous when expressed by deposition date rather than by position along the whisker. In Chapter 1, I proposed a method to improve deposition date estimates by applying individually adjusted growth rates and better estimates of shed date to wild harbor seal whiskers.

In Chapter 2, I analyzed stable isotope ratios from serial sections of whiskers of 32 harbor seals from a population that uses tidewater glacial habitats in southeast Alaska. I used a mixed-effects

repeated-measures model to determine the characteristics that influence stable isotope ratios over time. Mean stable carbon and nitrogen isotope ratios differed significantly among size classes ($p < 0.005$), with no effect of sex. Seals were then grouped by size to describe isotopic differences between different demographic groups using Standard Ellipse Corrected Area (SEA_c). Larger seals (>1.4 m) exhibited a broader isotopic niche in the fall, winter, and spring relative to smaller seals (< 1.4 m), but had a similar niche width in the summer. These results suggest that seals using tidewater glacial habitat share common prey base in the summer, while larger seals diversify their diets throughout the rest of the year. Overall, the results of this thesis suggest whisker morphometric characteristics can be used to improve the ability to make longitudinal inferences using serial sections of the whiskers, which reveal differences in prey utilization by size class in harbor seals that merit further study.

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Acknowledgements

This study would not have been possible without the generous support from many individuals and organizations. I would first like to thank my advisor, Diane O'Brien, for providing me the opportunity to join UAF as a graduate student, for taking a chance on me, and for her unwavering guidance and encouragement through my many lulls throughout. Shawna Karpovich, for her support through my time with the Alaska Department of Fish & Game, initiating this research, her countless hours discussing physiology, and support of my career as a wildlife biologist. My committee members Lara Horstmann for her guidance in the marine mammal sciences, invaluable comment on this thesis, and her general encouragement was truly meaningful to me. Greg Breed, for stepping in to fill an empty committee position, but more, he was always available for meetings and comments, and offered valuable insight of the analysis within. Perry Barboza, who was on my committee originally and provided sound advice and a great example of how a researcher should conduct oneself. Julie McIntyre for her willingness to meet, offer her invaluable statistical experience, and just generally being a positive influence in my time here at UAF. The whole stats department is truly an asset. I would also like to thank the Alaska Stable Isotope Facility (Norma Haubenstock and Tim Howe) for their advice and guidance for sample analysis, use of the lab facility, and good conversation while putting tissue in tin.

Financial and logistical support was provided by Alaska Department of Fish & Game through sample collection, providing me a job for several years, graduate award funding, and general valuable experiences. Jonathan Fiely and Jon Barton for being the best wingmen on seal team alpha, Christine Schmale for being a shining example of how a boss/person should carry out their business, and Gail Blundell for teaching many valuable lessons. I would also like to thank the National Fisheries Service and National Wildlife Research Fund for additional funding. Dan Foley of the M/V *Steller* was always a true teacher of how to work on a boat, which translates to how to work in life. And the whaler, that tank. Thank you for staying afloat.

Finally, I would like to proudly acknowledge the support of my mom, Sherry, and dad, Bill, who encouraged my career despite the fanatical penalty of doing what I love. My brother, Jared, for being a rad dude and forcing me to be a good example when I would rather not. My friends, near and far, that keep me in check when my horse gets a little high. My most faithful pal G. And most importantly, my best friend and partner Jen Curl. She makes the sun go round.

Introduction

Harbor seal (P*hoca vitulina*) populations range from 610,000 to 640,00 individuals worldwide (Bjørge et al. 2010) and are stable; thus, harbor seals are considered a species of least concern by the International Union for Conservation of Nature (Lowry 2016). However, some localized populations have faced declines or regional extinction due to viral outbreaks (Härkönen et al. 2006), bounty programs (Butler et al. 2008), or high levels of exposure to industrial and agricultural pollutants (Brouwer et al. 1989; Hoover-Miller et al. 2001; Reijnders 1986; Ross et al. 1995). In the northern hemisphere, sudden, substantial, temporally persistent reorganization of the ecological components in the marine environment are occurring (also known as a regime shifts) (Beaugrand et al. 2015; Hare and Mantua 2000), and include altered prey availability (Anderson et al. 1999; Hirons et al. 2001). Prior to the most recent regime shifts, overall populations of harbor seals in the Gulf of Alaska were relatively stable, with slight increases in Prince William Sound in recent years (Small et al. 2003). However, there have been sharp declines for harbor seals that use tidewater glacial habitat to haul out in the summer to molt, pup, nurse, and breed (Hoover-Miller et al. 2011; Mathews and Pendleton 2006; Womble et al. 2010). Although the causes of these population declines are not well understood, low-energy, lipid-poor prey may result in poor body condition and low reproductive success of seals, which ultimately has a negative effect on population size (Merrick et al. 1997; Tollit et al. 1997; Trites and Donnelly 2003). Gaining insight to the shifting seasonal diets of harbor seals will help us to understand the effects of local regime shifts on harbor seals in the Gulf of Alaska.

The diet of high trophic level predators such as harbor seals is difficult to evaluate due to the wide diversity of available prey items, the seals' high mobility, the logistic difficulties of capture, and the fact that seals spend a large amount of their life under water (Pitcher 1990). Satellite tracking, time depth recorders, and accelerometers are effective means to document foraging patterns by describing seal movements in their environment (Frost et al. 2001; Hastings et al. 2004; Lowry et al. 2001; Small et

al. 2005; Womble and Gende 2013). However, these technologies do not record prey consumed, and are often cost prohibitive and logistically challenging to deploy (Blundell et al. 2011; Bowen et al. 2002; Breed et al. 2009; Womble et al. 2014; Womble and Gende 2013). Analysis of prey hard parts in stomach and fecal contents provides a representation of what the seal was eating at a single point in time near the area of collection (Andersen et al. 2004; Bowen and Iverson 2013; Dehn et al. 2007; Geiger 2013; Lance et al. 2012; Tollit et al. 1998), but reflects recent diet only and is biased due to differences in digestion of different prey groups (Andersen et al. 2004; da Silva and Neilson 1985). Fecal DNA analysis can clarify digestion biases that occur with stomach or fecal hard part analysis and have a higher taxonomic resolution (Deagle et al. 2009), but still remain limited by time scale. While each of these methods is useful for specific questions, they are restricted by temporal resolution or cost.

For many marine mammal diet studies, stable isotopes of carbon and nitrogen are a widely used and are a relatively inexpensive method of chemical analysis to estimate trends in diet (Newsome et al. 2010). Stable isotopes are naturally occurring variations in the ratios of heavy and light isotopes (e.g., carbon and nitrogen; $^{13}C/^{12}C$ and $^{15}N/^{14}N$). Based on differences in diet and habitat, stable isotope ratios vary in the tissues of prey species (Burton and Koch 1999; Cherel et al. 2009; Newsome et al. 2010), and are commonly examined to assess how species use resources in their environment (DeNiro and Epstein 1978; Newsome et al. 2010). While stable isotopes cannot provide a taxonomic identification of prey, stable nitrogen isotope ratios are often utilized to assess trophic position, whereas stable carbon isotope ratios are applied to assess the marine habitat of the prey consumed, e.g. nearshore vs. offshore (Hobson 1999; Newsome et al. 2010). For example, Herreman et al. (2009) assessed stable isotope ratios in blood and hair to describe differences between age and sex classes of harbor seals that use both terrestrial and tidewater glacial habitats in the summer, and found that seals that use tidewater glacial haulouts forage on intertidal/demersal fish in the summer and had a higher degree of sexual segregation than seals that use terrestrial haulouts. Tissues with differing turnover rates, such as blood

plasma, red blood cells, muscle, and blubber offer insight into diet over periods of weeks to months (Beck et al. 2007; Herreman et al. 2009; Hobson and Clark 1992; Hobson et al. 1996; Lesage et al. 2001; Newsome et al. 2010). However, tissue turnover rates for pinnipeds are still largely understudied, and thus do not provide the temporal resolution needed to evaluate diet during the non-summer months (Hobson et al. 1996; Kurle and Worthy 2002). Unlike tissues that turn over, keratinous tissues, such as whiskers, can offer a higher resolution of dietary time series, because isotopic markers remain unchanged once deposited (Cherel et al. 2009). Whiskers have been used successfully to reconstruct the diet of pinnipeds, such as sea lions (Baylis et al. 2016; Lowther et al. 2011; Rea et al. 2015), fur seals (Cherel et al. 2009; Kernaléguen et al. 2012), and recently in a phocid (Hall-Aspland et al. 2005; Lowther et al. 2017; Lübcker et al. 2017)

Like most phocid whiskers, harbor seal vibrissae grow continuously throughout the year, fast at first and then slow, following an asymptotic curve described by the von Bertalanffy (1968) growth function (Beltran et al. 2015; Lübcker et al. 2016; McHuron et al. 2016; Sadou et al. 2014). Harbor seal whiskers are then shed following the slowest growth period sometime between May and August, once they have reached an asymptotic length (Hirons et al. 2001; Zhao and Schell 2004). Within the von Bertalanffy (1968) growth function, four parameters are required to estimate a temporal record of diet from phocid whiskers (Beltran et al. 2015; Lübcker et al. 2016; McHuron et al. 2016; Sadou et al. 2014). The parameters include (1) identifying when the whisker started growing (initiation date), (2) determining the growth rate of an individual whisker, (3) measuring the final whisker length (asymptotic length), and (4) estimating the number of days the whisker grows (lifespan). For some phocid species, these parameters are well defined (Beltran et al. 2015; Lübcker et al. 2016), and have allowed investigators to estimate seasonal shifts in diet using an asymptotic growth curve for phocid whiskers (Hall-Aspland et al. 2005; Lübcker et al. 2017). For harbor seals, only one group has assessed diet using stable isotopes in whiskers (de la Vega et al. 2017), but used linear growth values from Zhao and Schell

(2004) to estimate deposition date. Linear growth models estimate a deposition date similarly to non-linear models, when using a single value for growth, but they do not allow for variation in growth rates among different sized whiskers (Hall-Aspland et al. 2005; Rogers et al. 2016). Therefore, improvements on estimates of individual whisker growth rate and initiation date are needed to estimate stable isotope deposition date.

The overall goal of this thesis was to investigate whether the morphological features of harbor seal whiskers could be used to improve estimates of growth rate and keratin deposition date, and then use these methods to assess a record of diet over multiple seasons for a population of harbor seals that use tidewater glaciers for pupping and breeding. Past research has focused on the form and function of phocid whiskers (Ginter et al. 2012; Hanke et al. 2010; Miersch et al. 2011; Murphy et al. 2013), but not whisker morphology as it relates to growth. Most phocid whiskers have bumpy profiles, apart from bearded seals (*Erignathus barbatus*) and monk seals (*Monachus spp.*). In addition to the bumpy profile, whiskers that have been growing for the longest duration have a smooth section at the proximal end of the whisker (smooth root section; SRS), which has not been previously described. I hypothesized that (1) the SRS may be characteristic of the slowest growth rates as whiskers are reaching asymptotic length, (2) the bumpy section may be characteristic of faster growth rates, and (3) the mean distance between the bumps may be characteristic of individual whisker variability and therefore growth rates. In Chapter 1, I describe morphological features of the whisker to investigate whether whisker morphology could provide information on the rates and timing of whisker growth, and therefore improve the accuracy of translation of isotopic profiles along the length of the whisker into an estimated time series of diet.

In Chapter 2, I use the morphological whisker features described in Chapter 1 to translate position along the whisker into a deposition date for individual harbor seals using a non-linear growth model. This technique allowed me to use stable isotopes in the whiskers to estimate a record of seasonal changes in diet for a population of harbor seals in the Gulf of Alaska that pup and breed near

tidewater glaciers. I evaluate differences in stable isotope ratios over time between seals of different age and sex classes. I use the stable isotope data to compare the breadth and overlap for foraging niche for seals of different size and sex classes, to better understand the diversity of foraging within the populations. This information presents a useful tool for managers to assess trends in seasonal diet of harbor seals, to study conflicts and competition with commercial fishing and other marine predators, or to compare seasonal diet trends among years using archived whisker samples.

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Chapter 1. Morphological characteristics of harbor seal whiskers and their use in dietary reconstruction using stable isotope ratios[1]

1.1 Abstract

We investigated whether inferences about harbor seal (*Phoca vitulina*) whisker shed status and growth rate based on whisker morphology could improve dietary time series from whisker isotopic profiles. We identified morphometric characteristics of Alaskan harbor seal whiskers, including a smooth root section (SRS) and a bumpy section. The SRS was longer for spring-collected whiskers than fall-collected whiskers and matched the length of fully-grown, shed whiskers. Thus, SRS length can differentiate whisker shed and growth status, and we used it to determine the sequence of whisker shedding by cohort in summer-captured seals. The mean inter-bump length (IBL) correlated with whisker length, potentially providing a proxy for whisker growth rate. We compared stable carbon isotope ratio ($\delta^{13}C$) profiles along the three longest whiskers from 10 harbor seals, using two approaches for converting whisker position to date: (1) a standard growth rate constant based on captive studies and (2) individually adjusted growth rates based on whisker morphology. Intra-individual patterns of whisker $\delta^{13}C$ became more synchronous when expressed by deposition date rather than by position and individually-adjusting growth rates based on IBL did not improve synchrony further. These findings suggest that whisker morphology has the potential to contribute whisker growth rate and shedding information for dietary reconstruction.

[1] Smith, J., S. Karpovich, G. A. Breed, and D. M. O'Brien. Morphological characteristics of harbor seal whiskers and their use in dietary reconstruction using stable isotope ratios. Formatted for the Canadian Journal of Zoology.

1.2 Introduction

Like most phocids (true seals), harbor seals (*Phoca vitulina*) have complex foraging strategies and opportunistically switch prey at different times of the year (Bjorkland et al. 2015; Herreman et al. 2009; Newsome et al. 2010). Specialization on different prey by individual seals causes large variations in diet estimates, making generalization difficult (Herreman et al. 2009; Pierce et al. 1991; Pitcher 1980; Tollit et al. 1998). It would be useful to characterize harbor seal diet over the course of a year, for comparison among individuals, among years, and among populations. Such diet comparisons could provide evidence to understand the cause of dramatic changes in abundance in harbor seal population abundance across the Gulf of Alaska in the late 20th century (Boveng et al. 2003; Jemison et al. 2006; Pitcher 1990; Small et al. 2003), especially near areas with rapidly changing habitats, such as tidewater glaciers (Hoover-Miller et al. 2011; Mathews and Pendleton 2006; Womble et al. 2010).

Naturally occurring variations in the ratios of heavy and light carbon and nitrogen stable isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) are commonly used to assess how species use resources in their environment (DeNiro and Epstein 1978; Newsome et al. 2010). Based on differences in diet and habitat use, stable isotope ratios vary in the tissues of prey species (Burton and Koch 1999; Cherel et al. 2009; Newsome et al. 2010). These isotopes are incorporated into consumer tissues and do not change after deposition into the inert matrix of continuously growing structures, such as whiskers, baleen, claws, or teeth (Best and Schell 1996; Carroll et al. 2013; Walker and Macko 1999), allowing researchers to quantify consumer diet. Harbor seal whiskers contain dietary information across the seasons, because they grow throughout the year and are shed annually in the spring/summer (Hirons et al. 2001; Zhao and Schell 2004). However, phocid whisker growth rates vary over time (Hirons et al. 2001; Newland et al. 2011; Zhao and Schell 2004) and by location across the muzzle (Beltran et al. 2015; Greaves et al. 2004; McHuron et al. 2016). These sources of variability make it difficult to use stable isotope analyses of whiskers as a tool for dietary reconstruction. A better understanding of whisker growth rates and the

timing of whisker shedding would aid the use of stable isotope analysis from whiskers to generate annual dietary profiles for harbor seals.

Harbor seal whiskers have variable morphometric characteristics that may be used to assess growth status and identify the timing of stable isotope deposition into whisker tissue. Previous studies have shown that phocid whisker growth patterns follow an exponential distribution; fast growth at first and then slow to very low growth rates several months before being shed (Beltran et al. 2015; Lübcker et al. 2016; McHuron et al. 2016). Changes in whisker growth rates may be associated with the changes in morphology of harbor seal whiskers, specifically; (1) a smooth section at the proximal end of the whisker (not previously described and hereafter referred to as the "smooth root section", SRS) may be characteristic of the later, slowest growth rates, (2) the "bumpy section", with a sinusoidal form of repeating peaks and valleys for most phocids (Ginter et al. 2010; Hanke et al. 2010; Miersch et al. 2011) may be characteristic of faster growth, and (3) the mean distance between the bumps (hereafter referred to as the "inter-bump length", IBL) may be correlated with growth rate. Thus, we postulate that morphological characteristics can improve estimation of the deposition date of any given segment of whisker tissue in three ways. First, absence of the SRS may be used to identify whiskers that have not begun slow growth and therefore contain less temporal information. Second, SRS length may be used to identify how long the whisker has been growing slowly, thus allowing selection of similar whiskers for comparative analyses. Third, the IBL may be used to adjust the exponential growth rate of individual whiskers allowing comparison among whiskers that grew at different rates. How these morphometric characteristics relate to the rates of growth has not been studied in phocids.

The objective of this study was to investigate whether whisker morphology could provide information about the rate and timing of whisker growth, and improve the translation of isotopic profiles along the length of the whisker into an estimated time series of diet. First, based on the hypothesis that the length of the SRS corresponds to the duration of slower growth, and harbor seal

whisker shedding is estimated to occur late spring to early fall (Zhao and Schell 2004), we predicted: (1) whiskers from fall-captured seals (September 1 – October 30) would lack a distinct SRS; (2) whiskers from spring-captured seals (March 1 – May 30) would have a distinct SRS; and (3) summer-captured seals (June 1 – July 30) would have a bimodal distribution of whisker with and without an SRS, depending on whether whiskers were shed before or after capture. To validate these predictions, we compared SRS lengths of whiskers from each season with those of fully-grown whiskers shed naturally from captive harbor seals. We additionally assessed how SRS lengths were distributed over time in summer-collected whiskers, to estimate the timing of when whiskers were shed for each age and sex class. Second, we investigated whether SRS length, bumpy section length, and mean IBL were correlated. Correlations would suggest predictable variation among individual whiskers in growth rate, providing a tool for individually adjusting estimated dates of keratin deposition. We then tested if the decreasing IBL along the whisker from the tip to the root was correlated with position-specific growth rates measured in captive phocid studies (Beltran et al. 2015; Lübcker et al. 2016; von Bertalanffy 1968). Finally, we tested whether adjusting the growth rate equations using morphometric information from individual whiskers improved the synchrony of isotope profiles among multiple whiskers collected from the same individuals. Our aim was to improve the accuracy with which positions along a whisker can be translated into a timeline, allowing assessment of harbor seal diets year-round using stable isotope analysis.

1.3 Methods

1.3.1 Sample collection

We described the morphological characteristics of juvenile, sub-adult, and adult harbor seal whiskers (n=567) that were collected by the Alaska Department of Fish & Game (ADF&G). Harbor seals were captured in the Gulf of Alaska between March and October from 2003 to 2012. Capture locations

were Prince William Sound, Tracy and Endicott Arms, Glacier Bay, and Kodiak Island (Fig. 1). Seals were captured at terrestrial haul-out sites using multifilament seine nets and from iceberg haul-out sites using monofilament gillnets, both in accordance with ADF&G IACUC animal care protocols (#s 03-0015 and amendments #2012-12)). This work was conducted under NMFS permits (numbers 358-1787, 358-1585, 358-1757, and 16094).

Ages of the captured harbor seals were estimated from mass (kg) and curvilinear length (mm; along the contour of the back from tip of the tail to the tip of the nose) following Blundell and Pendleton (2008), and classified into age categories based on age at sexual maturity (Lydersen and Kovacs 2005; Pitcher 1990): juvenile = 0.75 to 1.75 years, sub-adult males = 1.75 to 5.75 years, sub-adult females = 1.75 to 3.75 years, adult males > 5.75 years, and adult females > 3.75 years. Seals were sedated with Diazepam (Hospira, Lake Forest, IL), injected intravenously at 0.25 mg kg^{-1} of body weight. Once seals were sedated, the longest available whisker was extracted with pliers to retain the entire shaft. Whiskers that broke during extraction were excluded from this study. In phocids, the longest whiskers are generally located at the most lateral position on the lower rows on the muzzle (Beltran et al. 2015; Greaves et al. 2004; Lübcker et al. 2016; McHuron et al. 2016; Sadou et al. 2014). Samples of shed (fully-grown) whiskers were collected from captive harbor seal enclosures at the Alaska SeaLife Center (ASLC) and compared to those extracted from wild-caught seals.

To examine whether whisker morphology improved synchrony of diet estimates when translating location along the whisker shaft to deposition date, patterns of stable isotope ratio variation were compared along three whiskers from each of 10 harbor seals collected by Alaska Native subsistence hunters near Yakutat, Alaska (N 59° 21'50", W 139° 22' 4") on March 26, 2003, and archived at the University of Alaska Museum (UAM) (UAM:Mamm 99535, 99536, 99537, 99538 99551, 99553, 99554, 99555, 99556, 99557). The UAM whisker collection protocol directs that the 8 longest whiskers

be removed from the muzzle of each seal; from these, we selected the 3 longest whiskers. These 10 seals were selected, because they were harvested on the same date from a single location.

1.3.2 Morphometric measurements of whiskers

To characterize the morphology of harbor seal whiskers, images were taken using a Nikon D50 camera with a NIKKOR 24-120 mm zoom lens mounted on a tripod. An amber light filter was placed over the flash and external lighting was used to increase image contrast. If necessary, whiskers were ironed flat using a common household hair straightener on the lowest heat setting and then photographed on a black felt background to minimize glare and maximize contrast. Digital images were measured using version 1.47 of ImageJ (Schneider et al. 2012). Digital images were first magnified, facilitating the accuracy of the very small morphological feature measurements. Points representing morphological features were then marked along the whisker shaft, and the number of pixels between each point was converted to mm by ImageJ (pixels per mm ranged from 141-396). A ruler was placed within the image to calibrate measurements between pictures. In the digital pictures, points were selected at the following locations (Fig. 3): (1) the root (proximal) end of the whisker; (2) the first distinguishable valley (i.e., the narrowing between two bumps); (3) the next distinguishable valley, and so on, until valleys were no longer distinguishable; and (4) the tip (distal) end of the whisker. The section between the root end of the whisker and the first distinguishable valley was termed the smooth root section (SRS). The section between the first distinguishable valley and the tip end of the whisker was termed the "bumpy section". The mean distance among valleys eight through 14 along an individual whisker was termed the mean inter-bump length (IBL). The middle six valleys were chosen, because (1) valleys were difficult to distinguish near the tip end due to abrasion, (2) IBLs closest to the SRS were uniformly small and uneven, and (3) IBLs 8-14 showed the most uniformity within a whisker and were most likely representative of the overall whisker length.

1.3.3 Stable isotope analysis

To prepare the 30 harbor seal whiskers for stable isotope analysis, whole whiskers were washed with 100% methanol, rinsed in deionized water in an ultrasonic bath for 30 minutes (to remove any dried blood and dirt), and then dried in a 60 °C oven for 30 minutes. Whiskers were placed under 13-mm-wide laboratory labeling tape and measured to the nearest mm. While under lab tape, whiskers were cut from the root end into one mm sections for the first 12 mm, and then cut into two mm sections from 12-20 mm. Beyond 20 mm, one mm sections were removed from the whiskers at 10 mm intervals, e.g. 20-21 mm, 30-31 mm, 40-41 mm, 50-51 mm, and so on to the tip end. Section widths often increased to 2-3 mm near to the tip to fulfill minimum sample weight requirements for stable isotope analysis (> 0.1 mg), as whiskers become thinner at their distal end (60-20 mm) (Fig. 3). Whisker sections were then cleaned with a 2:1 chloroform:methanol solution with sonication for 30 minutes, oven-dried for 30 minutes, rinsed in deionized water with sonication for 30 minutes, then oven-dried again. Whisker sections were placed in tin capsules and weighed with a microbalance; capsules were crushed and loaded into an autosampler for analysis.

Stable carbon isotope ratios were analyzed using a Finnegan Delta Plus XP isotope ratio mass spectrometer interfaced with a Costech ECS4010 Elemental Analyzer (Costech Scientific, Valencia, CA) at the University of Alaska Stable Isotope Facility in Fairbanks, AK. A calibration error occurred during the measurement of nitrogen isotope ratios, so we present results for carbon only. Results are presented in the conventional delta (δ) notation relative to the international standard for carbon (Vienna PeeDee Belemnite) using the equation:

$$\delta^{13}C\ [‰] = [{}^{13}C/{}^{12}C_{\ sample}/{}^{13}C/{}^{12}C_{\ standard}) - 1] \times 1{,}000‰$$

Precision was estimated using the laboratory peptone standards (n=15) run concurrently with whisker samples, which had a standard deviation of 0.22‰ for $\delta^{13}C$.

1.3.4 Analysis of morphometric characteristics

We tested whether the SRS length of whiskers from the wild-caught seals could be used to indicate growth status by comparing the SRS length between fall captured seals (Sept 1 – October 30) and spring-captured seals (March 1 – May 30), with the assumption that fall whiskers were rapidly re-growing following the summer shed, whereas spring whiskers were slowly and asymptotically approaching their final length. We used Analysis of Variance to test whether there was a difference in whisker SRS lengths between fall- and spring-caught seals with age class and sex included as independent variables. We then used the difference in SRS length between fall- and spring-collected seals to characterize the shed status of whiskers of summer captured seals (June 1 – July 30) by cohort. From the previous analysis we determined that the lower 95% confidence interval for the distribution of SRS lengths from spring-collected whiskers was 7.8 mm, which we then used as the threshold SRS length to discriminate summer-collected whiskers that were pre- and post-shedding. We used the distributions of SRS lengths in summer-collected whiskers to estimate the timing of whisker shedding for each age and sex class. A distinct SRS (≥ 7.8 mm) indicated that seals in each demographic group had not yet shed whiskers, an absent or indistinct SRS (< 7.8 mm) indicated recently shed whiskers, and bimodally distributed SRS would indicate active whisker shedding at the time of capture and whisker collection. We visually compared the probability density curves (from R density function; R Core Team 2013) of the SRS lengths for males and females separated by age class (i.e., adult, sub-adult, and juvenile). All statistical analyses were done in R version 3.0.2 (R Core Team 2013), and $\alpha \leq 0.05$ is considered statistically significant.

To investigate whether the SRS length, bumpy section length, and IBL were correlated in whiskers that were close to fully grown (≥ 7.8 mm SRS), we used reduced major axis regression to test if mean IBL of summer-collected whiskers (n = 98) predicted (1) the SRS length of a whisker close to fully

grown, and (2) the length of the bumpy section. We predicted that whiskers with the longest asymptotic lengths would have longer mean IBL's suggesting faster growth, longer SRS lengths, and longer bumpy sections.

1.3.5 Calculating deposition date from length

To determine when stable isotopes were deposited into the whisker segments, we used the von Bertalanffy (1968) growth function:

Equation 1 $$L_{t_i} = L_\infty \cdot (1 - e^{-K(t_i - t_0)}),$$

where length (L) at a given time (t_i) is found using a curvature constant (K), the asymptotic length (L_∞), and time at initial growth (t_0) (Beltran et al. 2015). To estimate deposition date, we first solved for time (t_i) at a given length;

Equation 2 $$t_i = \left[\frac{-1}{K} \cdot ln\left(1 - \frac{L_{t_i}}{L_\infty}\right)\right] + t_0$$

Because the data presented in this study indicate that shed dates vary among demographic groups, we allowed t_0 in Equation 2 to vary by cohort: August 10 (adult females), July 5 (subadults), and June 5 (juveniles)(ADF&G unpulished data 2017; Zhao and Schell 2004). In addition, we applied a length correction to March-collected whiskers to account for the difference between actual whisker length and expected asymptotic length, as follows: adult females ($L_\infty = measured\ L + 2.3\ mm$), sub-adult males and females ($L_\infty = measured\ L + 0.9\ mm$), and juvenile males ($L_\infty = measured\ L + 0.2\ mm$). We approximated the length correction for each cohort by estimating growth rate between actual collection date and t_0. A value for K (0.016) was based on past phocid whisker growth estimates (Beltran et al. 2015; Hall-Aspland et al. 2005; Lübcker et al. 2016; McHuron et al. 2016) and unpublished data on harbor seal whisker growth.

Previous studies demonstrated variability in growth rates among phocid whiskers, both within and among individuals (Beltran et al. 2015; Greaves et al. 2004; Hirons et al. 2001; Lübcker et al. 2016; McHuron et al. 2016; Newland et al. 2011; Zhao and Schell 2004). To account for differences in growth

rate among whiskers, we applied a correction factor to adjust the curvature constant (K) up or down based on morphology. This correction factor was calculated by dividing the mean IBL of an individual whisker (x_i) by the mean IBL of all whiskers ($\sum \frac{\{\bar{x}_i\}}{567} = 3.77\ m$) in the ADF&G collection ($x_i/3.77\ m$).

Equation 3 $$t_i = \left[\frac{-1}{\left(\frac{x_i}{3.77\ m} \cdot K\right)} \cdot ln\left(1 - \frac{L_{t_i}}{L_\infty}\right)\right] + t_0$$

To test if the individually adjusted growth function fit the changing distance between the IBL, we randomly selected 50 whiskers with a SRS from the ADF&G whisker archive and used linear regression to compare the IBL with the estimated growth rate of the whisker at each section. Growth rate estimates at each section were calculated by dividing the length of the whisker at a section, by the number of estimated days of growth (L_{t_i}/t_i).

We tested whether there was an improvement in the synchrony of stable isotope patterns along multiple whiskers from the same individuals when the position along the whisker was translated to dates between uniformly applied K, and K_i individually adjusted based on IBL. This was done in two steps: first, the mean $\delta^{13}C$ value was calculated within the months of June, September, and December separately for each whisker for each translation method, and second, the mean $\delta^{13}C$ value for all three whiskers per seal was calculated for each month. The mean difference among the mean of three whiskers and each individual whisker (per seal) for each selected month was compared between the two adjustment types using a student's *t*-test for June, September, and December. These months were chosen as examples of the different rates of whisker growth (fast, intermediate, and slow). In this test, we assumed that stable isotopes were deposited evenly across all whiskers, and that any difference is due to differences in growth rate and no other variable.

1.4. Results

1.4.1 SRS length

SRS lengths were significantly longer in spring-collected (16.7 ± 4.4 mm) compared to fall-collected whiskers (7.1 ± 4.2 mm; ANOVA, $F_{[4,383]} = 81.9$, $p < 0.0001$) (Fig. 4), while age class ($p = 0.73$) and sex ($p = 0.09$) were not significantly associated with SRS length. The mean SRS length of spring-collected whiskers of wild-caught harbor seals was not different from fully-grown, shed whiskers from captive seals (15.7 ± 2.8 mm; *t*-test, $p = 0.38$).

Figure 4 shows the distributions of whisker SRS lengths used to predict shedding status of summer whiskers by cohort. Few whiskers from adult males (33%, $n = 9$) had a distinct SRS. By contrast, whiskers from most (95%, $n = 23$) adult females had distinct SRS. Sub-adults follow a similar, but less pronounced trend, with a distinct SRS present in 46% of sub-adult males ($n = 35$) and 80% of sub-adult females ($n = 15$). For juveniles, roughly half of males (47%, $n = 47$) and females (57%, $n = 44$) had a distinct SRS.

1.4.2 Correlation of whisker morphometric characteristics

Among summer-collected whiskers with a distinct SRS (≥ 7.8 mm, $n = 98$), the mean IBL was strongly correlated with SRS length ($r^2 = 0.64$, $p < 0.0001$, Fig. 5A) and bumpy section length ($r^2 = 0.60$, $p < 0.0001$, Fig. 6B). Mean IBL from fully-grown whiskers from captive seals ($n = 17$) was also correlated with SRS length ($r^2 = 0.40$, $p < 0.0001$, Fig. 6A) and bumpy section length ($r^2 = 0.61$, $p < 0.0001$, Fig. 5B). The distributions of SRS lengths and mean IBL were similar between captive and wild-caught seals, but bumpy section lengths were longer in captive seals (Fig. 5A and 5B). Figure 6A shows an asymptotic relationship between the IBL and the position along the whisker (mm) from all whiskers, which seems to be similar in shape to the von Bertalanffy (1968) growth function. When testing this similarity among 50

randomly selected harbor seal whiskers with an SRS present, we found there was a strong correlation (r^2 = 0.61, p <0.0001) between the mean IBL and the estimated growth rate at each position (Fig. 6B).

1.4.3 Comparing isotopic profiles

Whisker $\delta^{13}C$ values varied along the length of individual whiskers, and patterns of variation were fairly consistent (synchronous) when compared across all three whiskers from an individual harbor seal (Fig. 7A). When we adjusted the position along the whisker to a deposition date using a uniform curvature constant (K), the synchrony of isotopic patterns among whiskers from the same individuals improved (for example, 99554 and 99551; Fig. 7B). Additionally, adjusting K by the IBL as shown in Equation 3 did not change the synchrony of $\delta^{13}C$ patterns vs. time (t-test; June, p = 0.94, September, p = 0.67, December, p = 0.15; Fig. 7C).

1.5 Discussion

Our study found that harbor seal whiskers that are close to fully grown, and approaching asymptotic length, can be identified by the presence of the SRS, a morphological feature that is reduced or absent in whiskers that are only partially grown. By using this feature to differentiate whisker growth status in summer-caught seals, we characterized the timing and relative progression of whisker shedding among cohorts. Additionally, this study found that the IBL changes over the length of whiskers in a pattern similar to the predicted whisker growth rates, and that total whisker lengths were positively correlated with the IBL. These findings indicate that the IBL may signify whisker growth rate at specific locations along the whisker shaft. When we used the von Bertalanffy (1968) growth function to translate position along the whiskers into deposition date, patterns in stable carbon isotope ratios became more synchronous among three whiskers from each seal. However, growth rate adjustments based on the mean IBL of individual whiskers did not improve the alignment of isotopic profiles within individuals due

to the similarity in morphometric characteristics among the longest 3 whiskers from each seal. The results of this study show that morphometric characteristics of harbor seal whiskers are highly correlated with the growth rates estimated using the von Bertalanffy (1968) growth function. However, there is unaccounted variation in individual whisker growth that should be explored, and controlled studies tracking changes in harbor seal whisker morphology and whisker growth rates are needed to refine temporal estimates at whisker positions.

Most phocids whiskers have bumpy profiles, although bearded seals (*Erignathus barbatus*) and monk seals (*Monachus spp.*) are notable exceptions, and past research has focused on the form and function of the bumps (Ginter et al. 2012; Hanke et al. 2010; Miersch et al. 2011; Murphy et al. 2013). However, none of these studies discussed the presence of an SRS, or that it varies seasonally. In this study, spring-collected whiskers had an SRS similar to naturally shed whiskers, fall-collected whiskers had a reduced or absent SRS, and summer-collected whiskers had a bimodal distribution of SRS lengths, as expected, because harbor seals molt pelage, and shed whiskers, during summer (Ashwell-Erickson et al. 1986; Daniel et al. 2003; Zhao and Schell 2004). For harbor seal researchers, a minimum SRS length of 7.8 mm, based on the lower 95% confidence interval of the spring-collected samples, could be used as a criterion for selecting whiskers that are approaching asymptotic length. Selecting older versus more recently grown whiskers will incorporate information over a longer period (i.e., stable isotope signatures) to estimate changes in diet over time.

When stable isotopes were plotted versus length for the three longest whiskers from 10 individuals, similar patterns in $\delta^{13}C$ values among whiskers from each individual were observed (Fig. 8A). These profiles aligned better than did the isotopic profiles in past phocid whisker studies (Hobson et al. 1996; Newland et al. 2011), perhaps because past studies did not use whisker root morphology to select whiskers with similar growth status. When the section of whisker representing the stable carbon isotope ratios was transformed to an estimated calendar date, the synchrony in the pattern improved in some

cases among whiskers from individual seals. We chose to compare stable carbon isotope ratios from the longest whiskers in this study to minimize the amount of variation introduced by differing shed dates, because the longest whiskers are located at the most caudal follicles on a phocid muzzle (Beltran et al. 2015; Greaves et al. 2004). Our findings demonstrate the importance of selecting a whisker that is close to fully grown with a distinct SRS and suggest that comparing whiskers with similar morphometric features will yield comparable results when assessing annual patterns in stable carbon isotope ratios among whiskers.

In the past, uncertainty surrounding the timing of whisker shedding limited the ability to use stable isotopes in serial sections of whiskers to estimate temporal shifts in diet (Greaves et al. 2004; Hirons et al. 2001; McHuron et al. 2016; Newland et al. 2011; Zhao and Schell 2004). Using the SRS lengths of the longest available whiskers as an indicator of growth status (i.e., close to fully grown vs. recently shed), we estimated the timing of whisker shedding for each harbor seal cohort in the summer months (June 1 – July 30). Our estimates of the timing of whisker shedding were limited by the dates that free-ranging seals were captured. However, our results suggest that most adult females retain their whiskers through at least late-July, while the majority of adult males shed their whiskers before June. Sub-adults follow this trend, but with a broader distribution of shedding times in both males and females. Juveniles were actively shedding their whiskers during our collection times, and there were no differences in shed timing between males and females. This sequence of whisker shedding in harbor seals also follows that of hair molt, where different harbor seal cohorts vary their peak molt times during the late summer (Ashwell-Erickson et al. 1986; Daniel et al. 2003; Thompson and Rothery 1987).

Phocid whiskers vary in length across the muzzle (Beltran et al. 2015; Greaves et al. 2004), suggesting differences in growth rate and function (Brecht et al. 1997; Miersch et al. 2011). Previous studies have shown that whisker length correlates with growth rate (Beltran et al. 2015; Lübcker et al. 2016; McHuron et al. 2016). We hypothesized that differing rates of growth would result in different SRS

lengths, bumpy section lengths, and mean IBLs. The strong correlations between these morphological characteristics and the comparison of the IBL, and growth rate at each position, supports our hypothesis that the mean IBL could be used as a morphological parameter to adjust growth rate estimates, because it is unaffected by wear at the tip of the whisker. When we applied a more complex transformation that used a standard growth rate individually adjusted based on the mean IBL, there were no further improvements to synchrony. However, most seals in our study showed similar IBLs across all three whiskers, which could indicate that whisker growth rates were already uniform. This result suggests that whiskers of similar length and morphology incorporate chemical signatures at similar times, as Lübcker et al. (2016) noted for elephant seals (*Mirounga leonine*).

Captive seals have different diets and behaviors that likely affect whisker growth and wear; therefore, whisker morphology may differ between captive and wild seals. As an example, we observed that the bumpy sections were longer in captive seal whiskers compared to those of wild-caught seals (Fig. 6), which may imply less wear. Our ability to translate stable isotope deposition date using the von Bertalanffy (1968) equations relies on measurement of total whisker length, which is affected by abrasion and wear. However, this study shows that IBL was correlated with the total length (bumpy and SRS lengths combined, Fig. 6), and therefore can be used to estimate total growth regardless of the amount of abrasion. Future research should investigate on how much wear occurs on harbor seal whiskers in the wild.

In conclusion, we have identified morphological features that can be used to differentiate a harbor seal whisker that is close to fully grown from one that is partially grown; specifically, a smooth root section that appears to be deposited during slower whisker growth. Using SRS length in whiskers collected during the summer months, we documented differences in whisker- shed timing by age class and sex. The length of the SRS combined with the IBL will allow researchers to visually separate partially grown whiskers from whiskers that are close to fully grown. This will allow for the selection of whiskers

that are most similar in growth status when examining stable isotopic signatures to construct a seasonal dietary profile. We estimated calendar dates of stable isotope deposition for harbor seal whiskers by applying previously reported phocid whisker growth rates and found that synchrony in stable isotope patterns among whiskers from the same individuals improved when expressed by date rather than by position (length). Adjusting the growth rate estimate using individual whisker morphology (mean IBL) did not improve the synchrony of stable isotope patterns over those based on a single uniform growth rate, due in part to the similarity among the longest whiskers selected. Future investigations of harbor seal diet using whisker stable isotope profiles should take morphology, specifically SRS length, into account to improve isotopic estimates of annual diet.

1.6 Acknowledgments

The authors thank the National Marine Fisheries Service (NMFS) for funding provided through congressional appropriations, the National Fish and Wildlife Foundation (grant #28217) for funding stable isotope analyses, and the Alaska Department of Fish & Game Graduate Award Program for additional support. This work was conducted under NMFS permit numbers 358-1787, 358- 1585, 358-1757, and 16094, and ADF&G Animal Care and Use Committee protocols (#s 03-0015 and amendments until 2012 (#2012-12). Additionally, the ADF&G staff and affiliates from 2003-2012 for sample collection and treasured experiences, specifically Christine Schmale, Gail Blundell, and Jon Barton; the ASLC for providing samples; the UAM for providing precious, non-duplicable samples; the Alaska Stable Isotope Facility (Tim Howe and Norma Haubenstock) for their direction and sample analysis; Julie McIntyre for her consistent willingness to lend her expertise, Lara Horstmann and Perry Barboza, for their guidance; Dan Foley of the M/V *Steller*; and the BIOL 604 class for invaluable comments.

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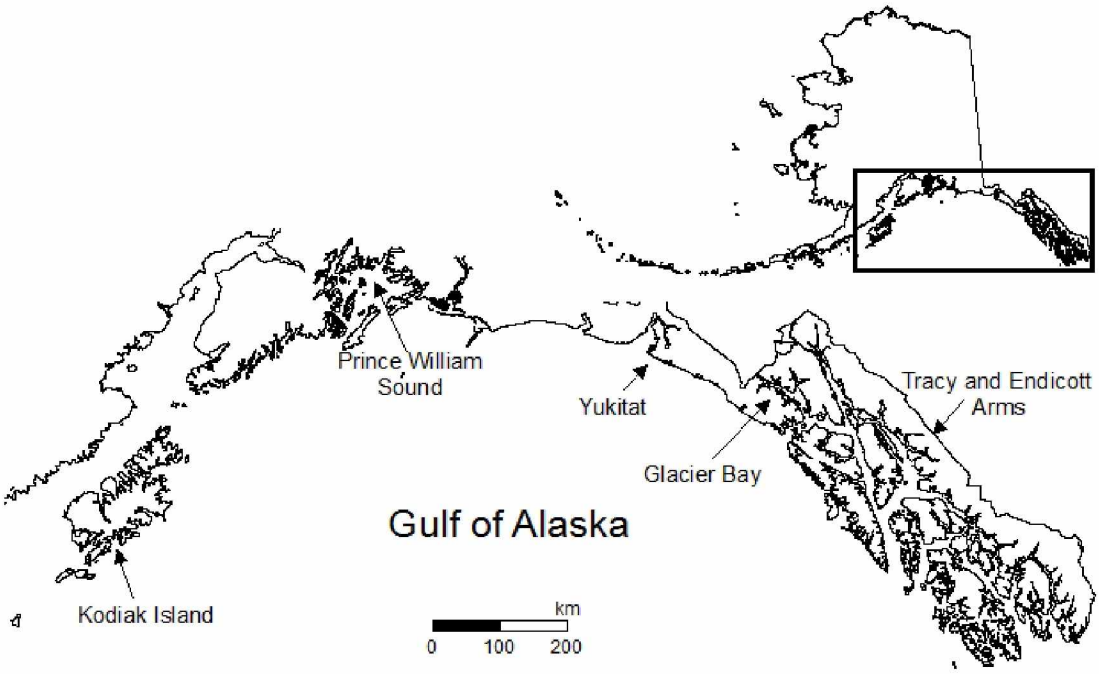

Figure 1.1. Harbor seal whisker collection sites in the Gulf of Alaska from wild-caught seals. Samples from Yakutat were obtained from Alaska Native subsistence harvests and were archived by the University of Alaska Museum; all others were live-captured by ADF&G.

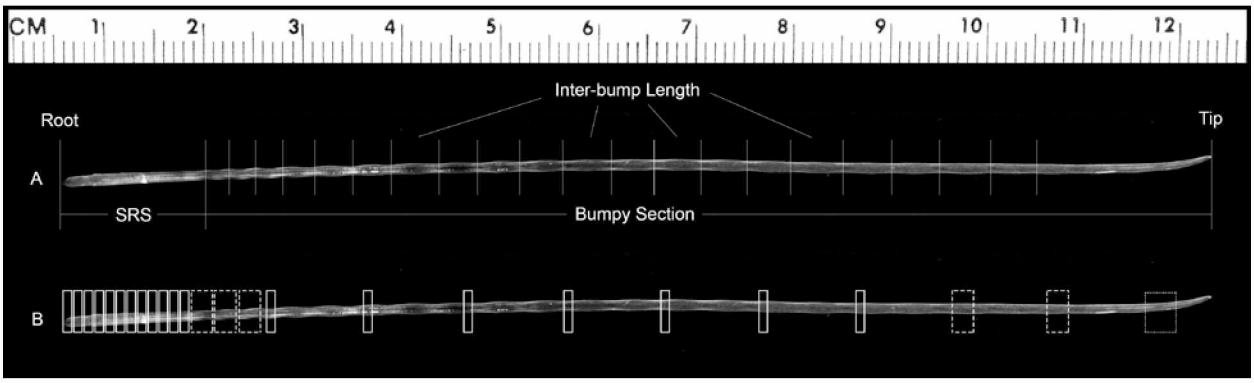

Figure 1.2. (A) Example of morphometric measurements obtained from individual harbor seal whiskers. All measurements were recorded from left to right with the root at 0 mm and included smooth root section (SRS) length, inter-bump length (IBL), and bumpy section length. (B) An example of serial sections from the whisker that were analyzed for stable carbon isotope ratios. White boxes represent 1 mm sections, dashed white boxes show 2 mm sections, and the gray box illustrates 3 mm sections.

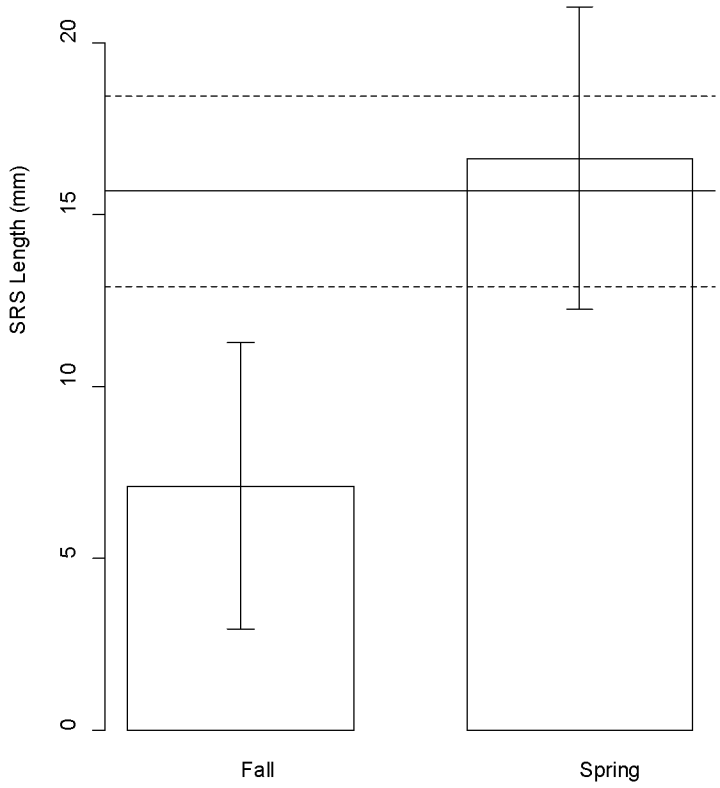

Figure 1.3. Mean (± 1SD) smooth root section (SRS) length (mm) of whiskers from harbor seals collected in fall (September 1 – October 30), and spring (March 1 – May 30); all sexes and ages combined (*n*=100). Horizontal line (± 1SD, dashed line) indicates mean SRS length of shed and presumably fully-grown whiskers from captive harbor seals (*n*=17).

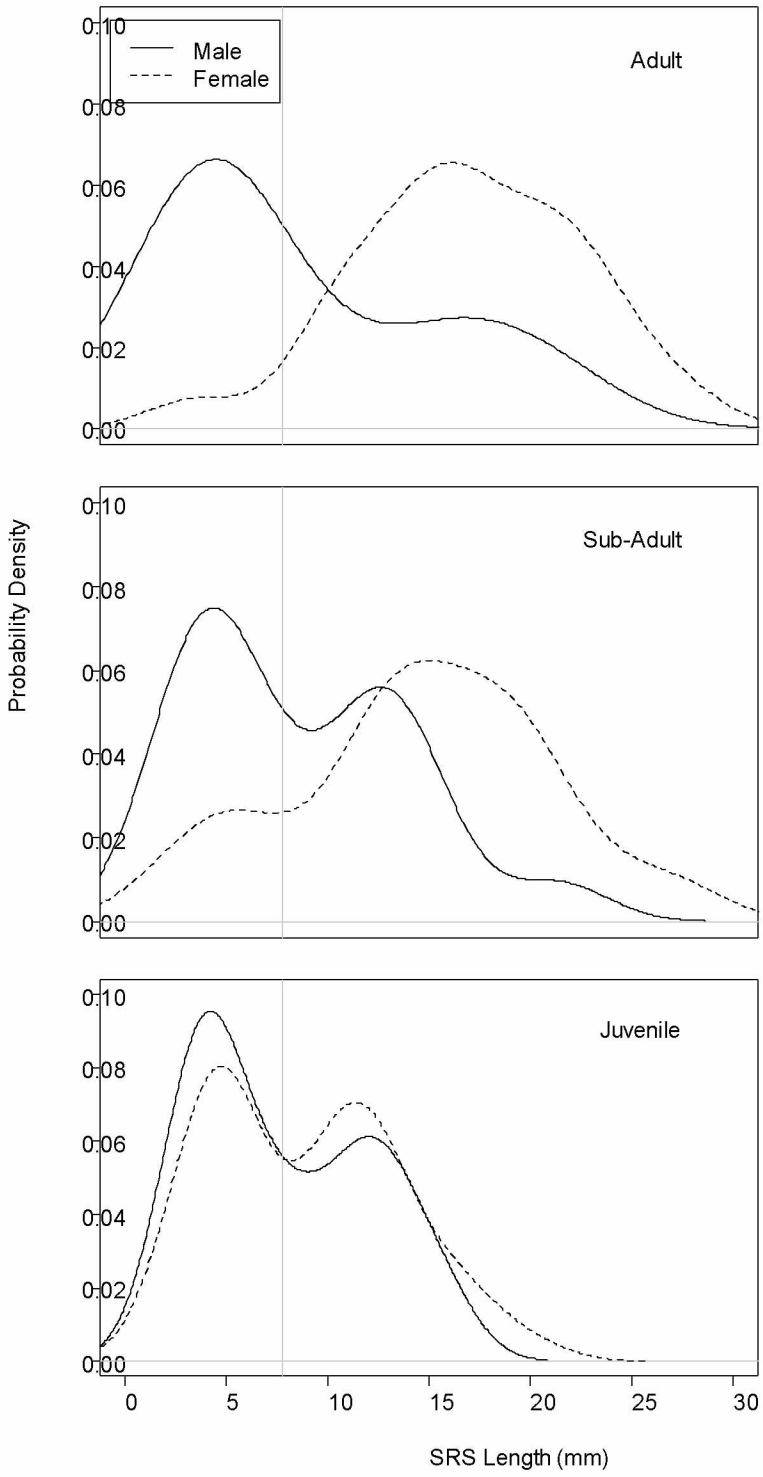

Figure 1.4. Probability density shows the frequency of distribution of the smooth root section (SRS) length (mm) for summer-collected (June 1 – July 30) harbor seal whiskers by age class and sex. There is a bimodal distribution of SRS lengths above and below approximately 7.8 mm (vertical gray line). An SRS length of less than 7.8 mm suggested that the whiskers was close to fully grown, and an SRS length greater than 7.8 mm suggests that the whiskers were at or near fully-grown.

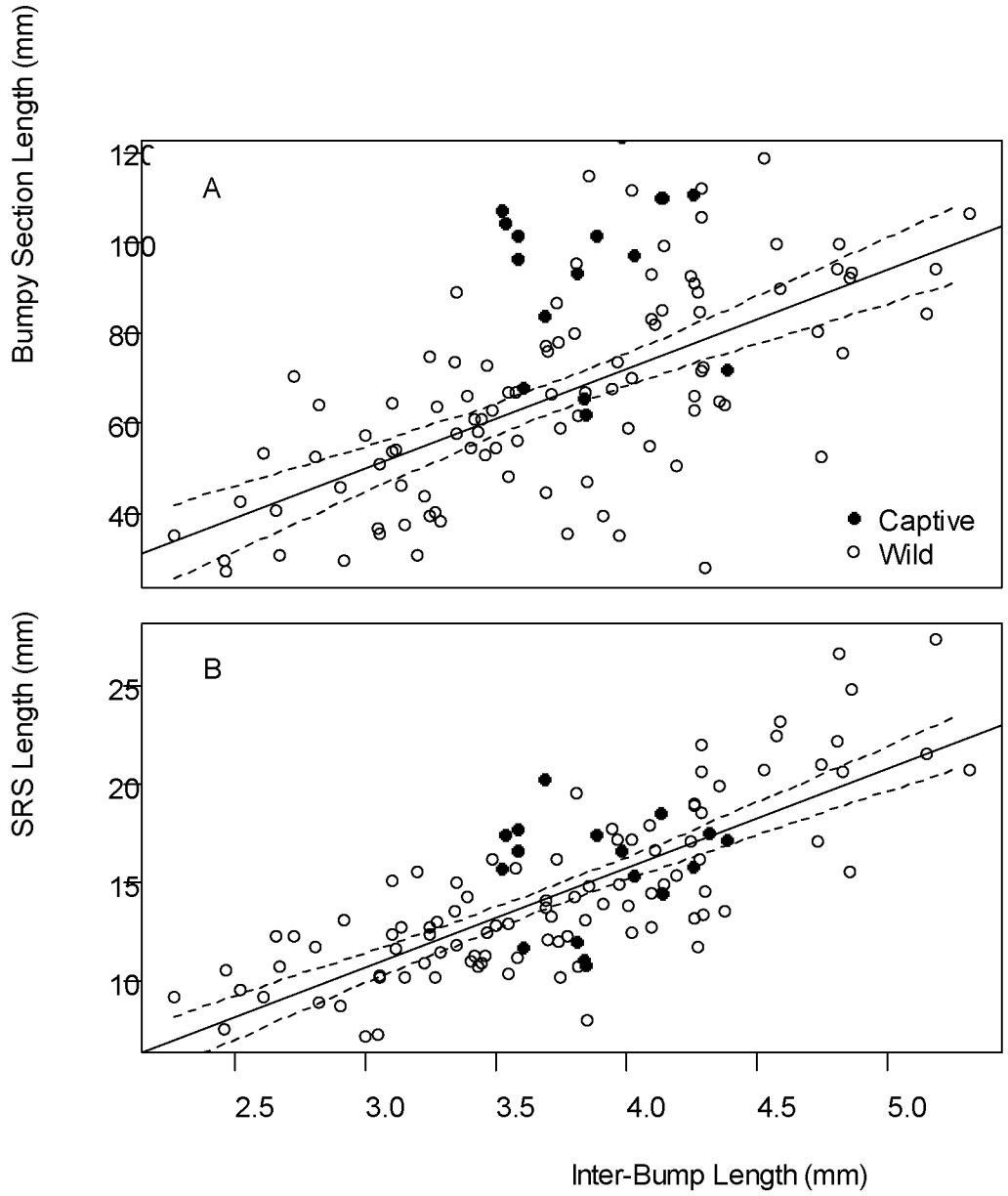

Figure 1.5. Reduced major axis regression of the mean distance among valleys eight through 14 (x) with length of the smooth root section (SRS) length (n = 98, r^2 = 0.63, p < 0.0001) and the bumpy section (n = 98, r^2 = 0.63, p < 0.0001) for all nearly fully-grown summer-collected whiskers (open symbols). The solid line represents the mean regression line, and dashed lines represent the 95% CI for the slope of the line. Fully-grown whiskers, shed from captive seals (n = 17), are included for comparison (filled symbols). Whiskers that lack a distinct SRS (< 7.8 mm) from the summer were excluded.

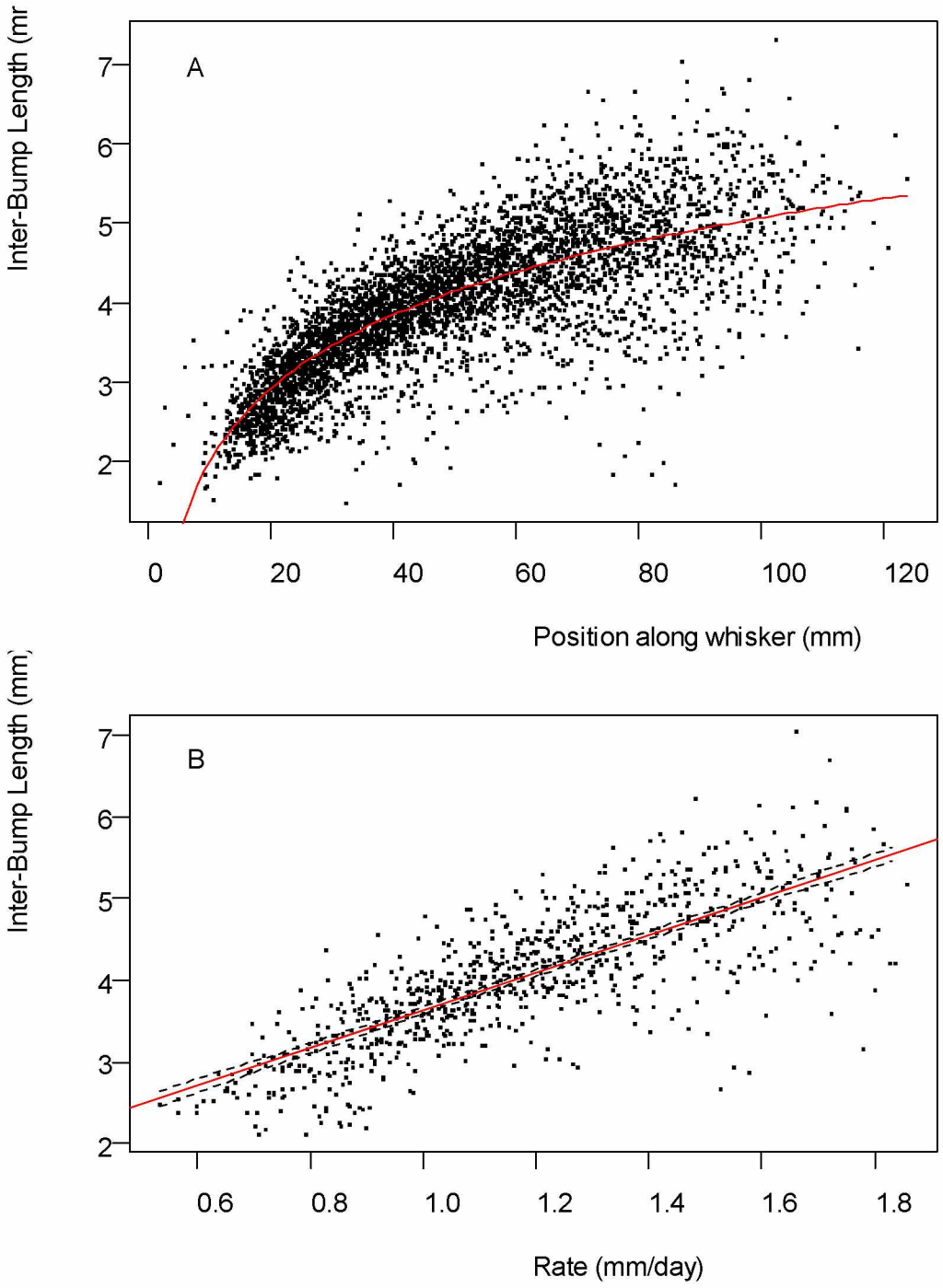

Figure 1.6. (A) Inter-bump length (IBL) versus the position along the whisker (mm) from whiskers collected from live-captured wild seals. Whiskers selected were from adult, sub-adult, and juvenile harbor seals with a distinct SRS (n = 442). (B) Linear regression of IBL along the whisker and growth rate (mm/day) at each position for randomly selected harbor seals in the ADF&G whisker archive with a distinct SRS (n = 50, r^2 = 0.65, p < 0.0001). The red line represents the mean regression line, and gray lines represent the 95% CI for the slope of the line.

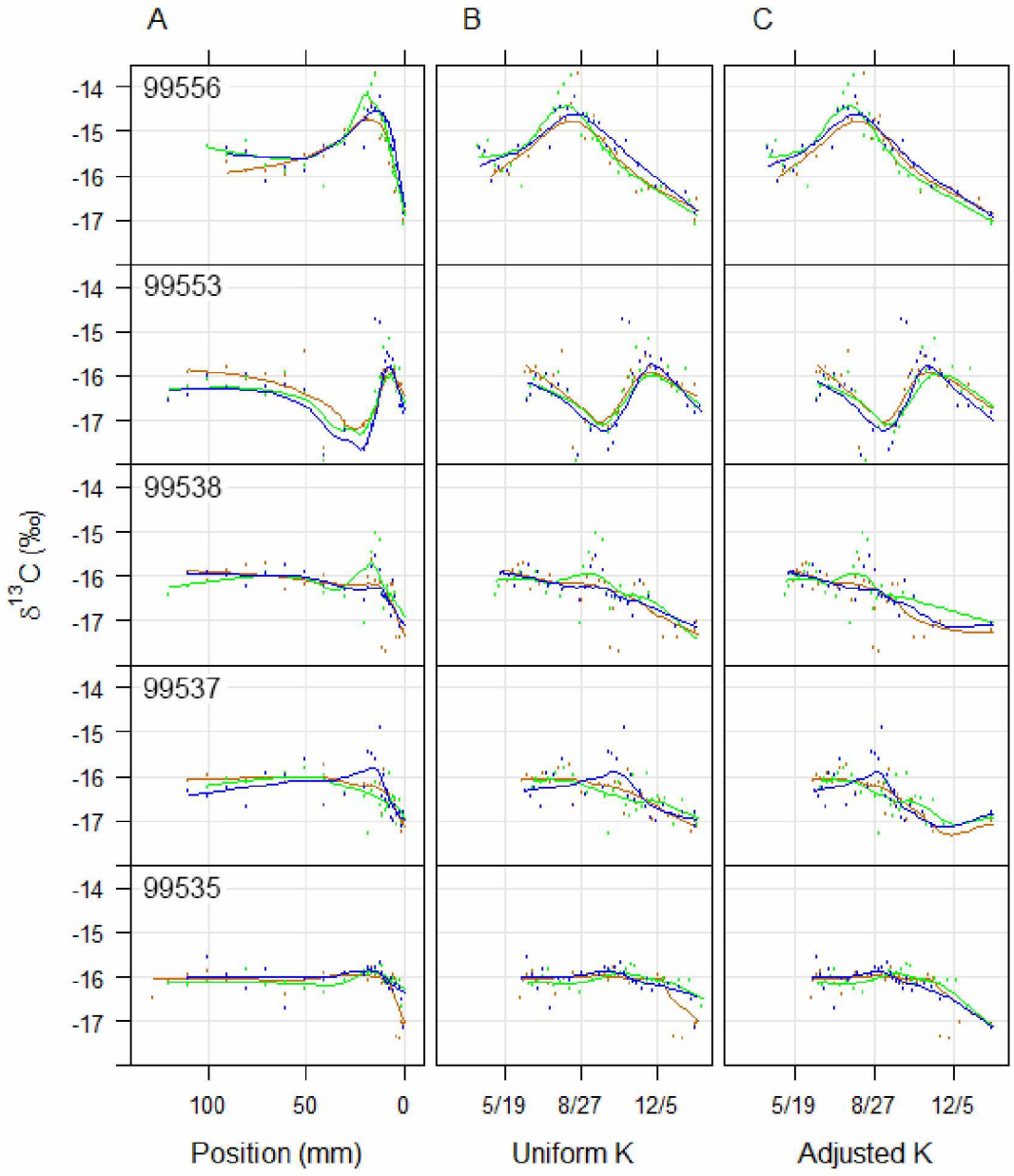

Figure 1.7 (A) Carbon stable isotope ratios ($\delta^{13}C$ values) versus length for the three longest whiskers of 10 harbor seals collected at the same location (Yakutat, AK) and on the same date (March 26, 2003). The x-axis represents distance from the root end in mm. (B) $\delta^{13}C$ values versus estimated calendar date, based on estimates of growth rate and the smooth and bumpy section lengths (Equations 1 & 2). The x-axis represents days prior to the date of collection (March 26, 2003). (C) $\delta^{13}C$ values versus calendar date, based on individually adjusted growth rate estimates (Equations 3 & 4) and the smooth and bumpy section lengths. Each line represents one whisker, with three whiskers analyzed for 10 seals. All samples were provided by University of Alaska Museum, denoted by UAM:MAMM identification number.

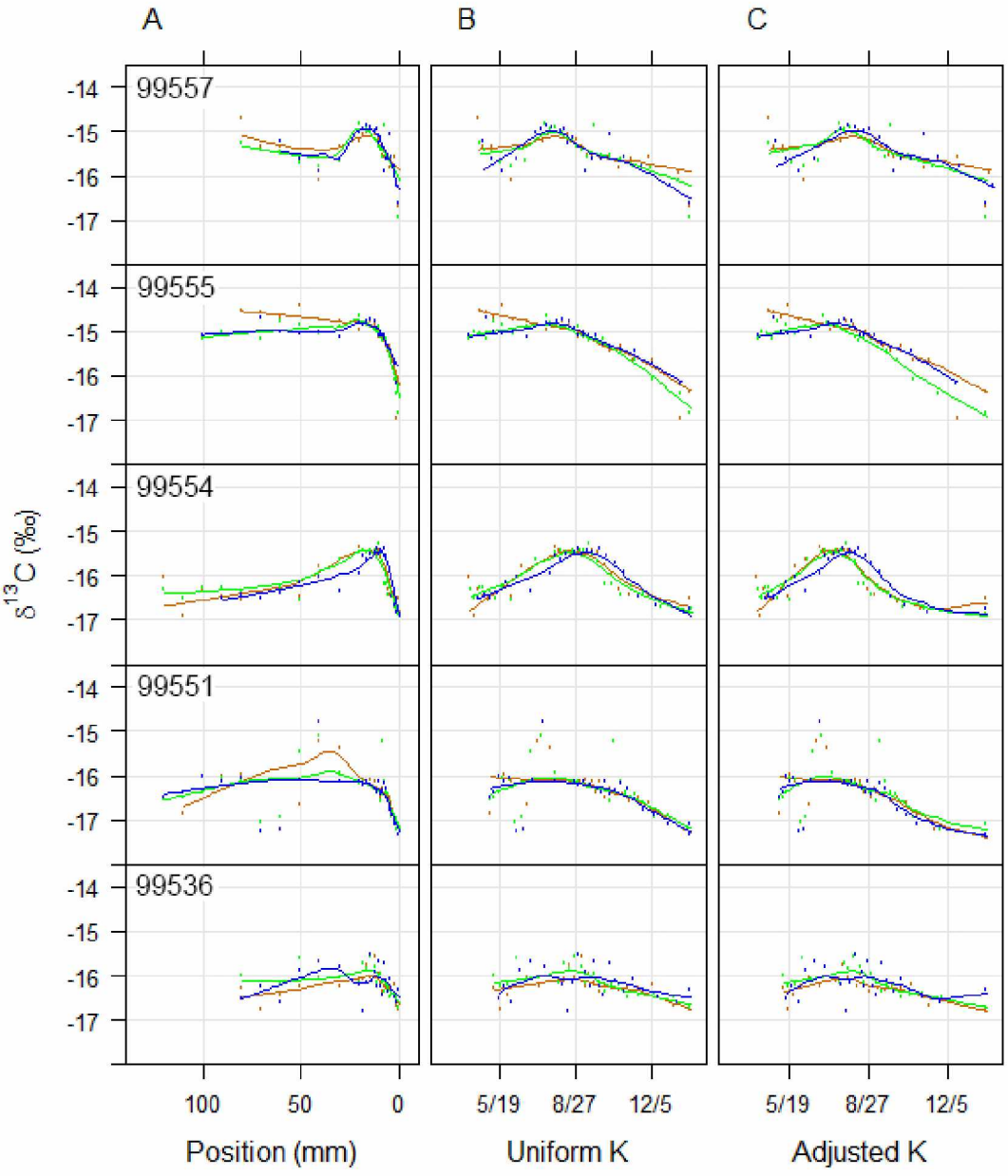

Figure 1.7 (cont.)

Chapter 2. Seasonal differences in foraging and isotopic niche width are related to size in Gulf of Alaska harbor seals[2]

2.1 Abstract

Harbor seals (*Phoca vitulina*) use different foraging strategies based on size and sex. Here, we used longitudinal stable isotope analysis of whiskers to assess seasonal foraging of seals using tidewater glacial habitat in southeast Alaska. We analyzed stable isotope ratios from serial sections of 32 harbor seal whiskers and estimated a deposition date for each serial section. We then used a mixed-effects repeated measures model to determine the characteristics that influence stable isotope ratios over time. We grouped seals by size to describe isotopic differences between different demographic groups using Standard Ellipse Corrected Area (SEA_c). Mean carbon and nitrogen isotope ratios differed significantly between size classes ($p < 0.005$), with no effect of sex. Larger seals (>1.4 m) exhibited a broader isotopic niche in the fall, winter, and spring relative to smaller seals (< 1.4 m), but not in the summer. This suggests that seals using tidewater glacial habitat share common foraging behavior in the summer, while larger seals exhibit more diverse foraging patterns throughout the rest of the year. These results highlight the importance of tidewater glacial habitat for all harbor seals during the summer months.

[2] Smith, J., S. Karpovich, L. Horstmann, J. McIntyre, and D. M. O'Brien. Seasonal differences in foraging and isotopic niche width are related to size in Gulf of Alaska harbor seals. Formatted for the Canadian Journal of Zoology.

2.2 Introduction

Harbor seals (*Phoca vitulina*) that haul out on floating ice near tidewater glaciers in the summer to pup, nurse, breed, and molt make up 10% of all Alaskan harbor seals (Bengtson et al. 2007), and are considered to be important source populations in the Gulf of Alaska because of the unusually high proportion of pups to mothers when compared to seals that use terrestrial haulouts (Calambokidis et al. 1987; Womble et al. 2010). However, harbor seal populations that use floating ice near tidewater glaciers in southeast Alaska have sharply declined (Mathews and Pendleton 2006; Womble et al. 2010), while seal populations that use terrestrial haulouts in southeast Alaska seem to be stable (Small et al. 2003). The reason for the difference in population status is unknown. One behavioral difference between these two populations is that glacial seals travel farther and dive deeper for longer foraging bouts than terrestrial seals (Blundell et al. 2011; Womble et al. 2014). When seals that use glacial habitat are not foraging, floating glacial ice offers a reliable platform for longer haulouts, less disruptions from tides, and a milder sea and weather state (Calambokidis et al. 1987). Previous work has described the diet of glacial harbor seals during the summer pupping and breeding season in the Gulf of Alaska (Blundell et al. 2011; Herreman et al. 2009; Womble et al. 2014); however, it remains unknown during other seasons. We aim to estimate temporal records of diet across all seasons to evaluate when harbor seals that haul out near tidewater glaciers target key prey groups.

Previous studies of harbor seal diet have examined only short time periods that are generally limited to summer when seals are easily accessed (Beck et al. 2007; Newsome et al. 2010), or spatial monitoring studies that require expensive instrumentation (Cherel et al. 2009; Hirons et al. 2001; Zhao and Schell 2004). Short-term diet analyses include, but are not limited to: (1) chemical analysis of tissues that metabolically turn-over (e.g., muscle, blubber, and blood) (Beck et al. 2007; Herreman et al. 2009; Hobson and Clark 1992; Lesage et al. 2001; Newsome et al. 2010), (2) fecal and stomach content analysis, which can be biased due to differences in prey digestibility (Andersen et al. 2004; Bowen and

Iverson 2013; Dehn et al. 2007; Geiger et al. 2013; Lance et al. 2012; Tollit et al. 1998), (3) chemical analysis of bone, teeth, or claws, which can be invasive or require post-mortem tissue collection (Aubail et al. 2010; Carroll et al. 2013; Hirons et al. 2001; Newsome et al. 2010; Seymour et al. 2014; Sheffield and Grebmeier 2009), and (4) remote tracking (GPS and VHF) to understand foraging locations among and within populations (Blundell et al. 2011; Bowen et al. 2002; Breed et al. 2009; Womble et al. 2014; Womble and Gende 2013), but is costly and limited by the number of seals that can be tracked. A cost-effective method is needed to estimate long-term diet patterns in harbor seals.

Analysis of stable isotopes from serial sections of harbor seals whiskers allow us to estimate a temporal record of diet for up to a year, because phocid whiskers grown continuously and are composed of keratin, which is metabolically inert (Cherel et al. 2009; Hirons et al. 2001; Hobson et al. 1996; Zhao and Schell 2004). Stable isotope ratios vary among prey species according to trophic position and sources of primary productivity (Hobson 1999; Newsome et al. 2010), and the ratios are preserved in the growing tissues of consumers (DeNiro and Epstein 1978). Measuring serial sections of whiskers for naturally-occurring variations in the ratios of heavy and light carbon and nitrogen stable isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) will allow us to quantify consumer diet over time. The isotopic niche width quantifies the dispersion of stable isotope ratios among individuals within a population, and is interpreted as a measure of consistent intra-population differences in foraging pattern (Bearhop et al. 2004; Jackson et al. 2011; Newsome et al. 2007). Phocid whisker growth rates are described by a von Bertalanffy (1968) growth function, with initial fast growth, then slow growth to reach final asymptotic length prior to shedding (Beltran et al. 2015; Greaves et al. 2004; Lübcker et al. 2016) in the summer (Chapter 1; Zhao and Schell 2004). Our current understanding of harbor seal whisker growth and variability (Chapter 1) allows us to describe seasonal isotopic niche width for a harbor seal population from serial sections of harbor seal whiskers.

In this study, we used stable isotope ratios from serial sections of harbor seal whiskers to assess seasonal dietary trends for a population of seals that use tidewater glacial habitat in the summer. To do this, we (1) analyzed the $\delta^{13}C$ and $\delta^{15}N$ values of serial whisker sections and applied a harbor seal-specific whisker growth curve to translate whisker position into a timeline of stable isotope deposition, (2) determined if harbor seal size and sex influence seasonal dietary trends, and (3) characterized dietary overlap between seal size classes in each season by assessing isotopic niche width. Finally, we used isotopic niche width to characterize the seasonal trends in harbor seal diets, and investigated how sex and size affect those seasonal trends.

2.3 Methods

2.3.1 Sample collection

We selected whiskers from the Alaska Department of Fish and Game's (ADF&G) collection of harbor seal tissues obtained in the Gulf of Alaska. All seals were captured under NMFS Research Permits #358-1787 and 358-1585 using active and passive netting techniques (Herreman et al. 2009) in accordance with ADF&G IACUC protocols (#s 03-0015 and amendments #2012-12). If necessary, seals were sedated with Diazepam (Hospira, Lake Forest, IL), injected intravenously at 0.25 mg kg^{-1} of body weight. Body morphometric measurements were collected and included mass (kg), curvilinear length (m) (tip of the tail to the tip of the nose), and axial girth (m) (circumference measurement behind front flippers). For all seals captured, the longest available whisker was extracted with pliers to retain the root. The longest whisker is typically located at the most lateral portion on the lower rows on the muzzle (Beltran et al. 2015; Lübcker et al. 2016; McHuron et al. 2016). Images of whiskers were taken using a Nikon D50 camera with a NIKKOR 24-120 mm zoom lens mounted on a tripod, and measured using ImageJ (Schneider et al. 2012), following methods previously described (Chapter 1).

All harbor seal whiskers in the ADF&G whisker collection were collected in the Gulf of Alaska between 2002 and 2014 (Fig 1). For this study, we used four criteria to select whiskers from the ADF&G whisker archive: (1) collected in Tracy and Endicott Arms in the summer of 2009 or 2010, (2) presence of a long smooth section of the whisker near the root, which indicates that the whisker was close to fully grown (Chapter 1); (2) collected in the summer, when whiskers are close to fully grown (Chapter 1); and (3) sampling males and females as evenly as possible (19 females and 13 males; Table 1). Few large male seals were captured, and they are therefore under-represented in our analysis.

2.3.2 Stable isotope analysis

Thirty-two whiskers were prepared for stable isotope analysis. Whole whiskers were washed with 100% methanol, sonicated in deionized water for 30 minutes to remove any dried blood and dirt, and dried in a 60 °C oven for one hour. Whiskers were placed under 13-mm-wide laboratory labeling tape and measured to the nearest mm. While under lab tape, whiskers were cut from the root end in one mm sections at approximately three mm intervals (e.g., 0-1 mm, 4-5 mm, 7-8 mm, 10-11 mm), increasing to ten mm intervals in the more distal, rapidly growing section (e.g., 20-21 mm, 30-31 mm, 40-41 mm, 50-51 mm, etc.). Section widths often increased to 2-3 mm near the tip to fulfill minimum sample weight requirements (> 0.1 mg), as the whiskers become thinner at their distal end (60-120 mm) (Fig. 3). Whisker sections were then sonicated in a 2:1 chloroform:methanol solution for 30 minutes, and oven-dried, sonicated in deionized water for 30 minutes, then oven-dried again. Individual sections were placed in tin capsules and weighed with a microbalance, capsules were then crushed and loaded into an autosampler for analysis.

Stable isotope ratios of carbon and nitrogen were analyzed using a Finnegan Delta Plus XP isotope ratio mass spectrometer interfaced with a Costech ECS4010 Elemental Analyzer (Costech Scientific, Valencia, CA) at the University of Alaska Fairbanks Stable Isotope Facility. Results are

presented in the conventional delta (δ) notation relative to international standards for carbon and nitrogen using the equation

$$\delta X\ [‰] = [R_{sample}/R_{standard}) - 1] \times 1{,}000‰$$

with X = the heavy isotope (^{13}C or ^{15}N) and R = the isotope ratio ($^{13}C/^{12}C$ or $^{15}N/^{14}N$). Vienna PeeDee Belemnite was used as a standard for carbon and N_{AIR} for nitrogen. Precision (SD ≤ 0.08‰ for both $\delta^{13}C$ and $\delta^{15}N$) was estimated using peptone standards (n=15) run concurrently with whisker samples.

2.3.3 Determining deposition date

To estimate when stable isotopes were deposited into the whisker segments, we used the von Bertalanffy (1968) growth function:

Equation 1 $$L_{t_i} = L_\infty \cdot (1 - e^{-K(t_i - t_0)}),$$

where length (L) at a given time (t_i) is found using a curvature constant (K), the asymptotic length (L_∞), and time at initial growth (t_0) (Beltran et al. 2015). To estimate deposition date, we first solved for time (t_i) at a given length;

Equation 2 $$t_i = \left[\frac{-1}{K} \cdot ln\left(1 - \frac{L_{t_i}}{L_\infty}\right)\right] + t_0$$

To satisfy the requirements of this equation and to simplify our analysis, we made two assumptions about whisker growth: (1) whiskers collected from seals in the summer are at or near asymptotic length ($L_\infty = L$ at $t_{collect}$) because whiskers are typically shed at the end of the summer; (2) dates that whiskers began growing (t_0) are accurate for each whisker based on the size and sex of the seal (Chapter 1; Zhao and Schell 2004). We used an initial base value for K (0.016), which was based on past phocid whisker growth estimates (Beltran et al. 2015; Hall-Aspland et al. 2005; Lübcker et al. 2016; McHuron et al. 2016) and unpublished data on harbor seal whisker growth (ADF&G unpulished data 2017). To individually adjust whisker growth, we assigned an individually adjusted value for curvature constant ($K \cdot x_i/3.77\ m$) for each whisker as described in Chapter 1. Briefly, the method uses a modifier of K based

on the middle six "inter-bump length" measurements (x_i), which was found to be positively associated with individual whisker length and growth rate (Chapter 1).

2.3.4 Linear mixed-effects models

Linear mixed-effects (LME) models were used to explain how $\delta^{13}C$ and $\delta^{15}N$ values change over time, and how such changes are associated with seal size and sex characteristics. Models were fit separately for $\delta^{13}C$ and $\delta^{15}N$ values using the lme4 package version 1.0 (Bates et al. 2014) for R (R Core Team 2013). Models estimated parameters describing the fixed, overall population trend in isotope values over time, as well as random effects describing random variation in that trend among individual seals in the population (n = 32). The additional fixed effects of sex, size and capture year were included in models to investigate differences in the overall time trend among different groups.

Our study collected a number of morphological measurements that could be used to describe seal size, namely body curvilinear length, axial girth, and weight. Because these variables are all highly correlated, only one was included in the analysis, and initial modeling efforts focused on identifying the one that was most associated with each response. A reduced model that included only time effects was compared to models that included time effects as well as one of the three morphological covariates. Likelihood ratio tests computed with the R package lmerTest (Kuznetsova et al. 2015) were used to compare models. The morphological variable with the lowest p-value was selected and included in subsequent investigation of the remaining fixed and random effects. The best-fitting models were chosen for both $\delta^{13}C$ and $\delta^{15}N$ values using the Akaike information criterion (AICc) value (Table 2). Residual plots were used to assess the assumptions of normality and homoscedasticity.

2.3.5 Niche width comparison

To describe temporal trends in stable isotope ratios of harbor seal whiskers, seals were separated into two categories by curvilinear length, large (≥ 1.4 m) and small (< 1.4 m). These categories were chosen because both male and female harbor seals reach sexual maturity at a curvilinear length near 1.4 m (Blundell and Pendleton 2008). To test the seasonal dietary differences in trophic niche width of harbor seals from the two size classes we used the Stable Isotope Bayesian Ellipses in R (SIBER) package (Jackson et al. 2011; R Core Team 2013). We calculated mean $\delta^{13}C$ and $\delta^{15}N$ values for seals in each season, defined as: summer09 = 4/30/09 – 7/20/09, fall09 = 7/21/09 – 10/28/09, winter09 = 10/29/09 – 2/5/10, spring10 = 2/6/10 – 5/16/10, and summer10 = 5/17/10 – 7/14/10. The SIBER package uses a Bayesian framework that estimates the size and shape of a generated 95% confidence interval Standard Ellipse Corrected Area (SEA_c), which is represented as the population's isotopic trophic niche width (Jackson et al. 2011). We compared niche width (SEA_c values) between large and small seals. We chose this method over convex hull area because the convex hull area method requires an unfeasibly large sample size to describe the isotopic niche width of a population (Jackson et al. 2011; Syväranta et al. 2013).

2.4 Results

2.4.1 Linear mixed-effects models

Of the body morphology parameters tested in our LME models, harbor seal curvilinear length had a larger and more significant effect on the trends (± se) in $\delta^{13}C$ ($\hat{\beta}$ = 0.02 ± 0.006; p = 0.009) and $\delta^{15}N$ ($\hat{\beta}$ = 0.02 ± 0.008; p = 0.005) values, compared with body mass ($\delta^{13}C$; p = 0.076 and $\delta^{15}N$; p = 0.025) and axial girth ($\delta^{13}C$; p = 0.270 and $\delta^{15}N$; p = 0.119). Therefore, we selected curvilinear length to describe body size of each seal, and it was included in subsequent models of whisker stable isotope ratios.

Whisker $\delta^{13}C$ values showed a significant positive linear correlation with time ($\hat{\beta}$ = 0.010 ± 0.001; $p < 0.001$), with a negative quadratic component ($\hat{\beta}$ = - 1.6e^{-5} ± 1.9e^{-6}; $p < 0.001$), increasing slightly through the fall and winter and then decreasing through the following summer. By contrast, $\delta^{15}N$ values exhibited a significant negative linear correlation with time ($\hat{\beta}$ = - 0.007 ± 0.001; $p < 0.001$), with a positive quadratic component ($\hat{\beta}$ = 1.6e^{-5} ± 2.0e^{-6}; $p < 0.001$), decreasing through the fall and winter and increasing through the following summer. The random effect of individual seal was significant for both the intercept ($\delta^{13}C$; σ_1^2 = 0.947; $\delta^{15}N$; σ_1^2 = 0.624) and the slope ($\delta^{13}C$; $\hat{\sigma}_0^2$ = 6.5e^{-6}; $\delta^{15}N$; $\hat{\sigma}_0^2$= 2.3e^{-6}) in whisker $\delta^{13}C$ and $\delta^{15}N$ values. Capture year ($\delta^{13}C$; p = 0.979 and $\delta^{15}N$; p = 0.296) and sex ($\delta^{13}C$; p = 0.728 and $\delta^{15}N$; p = 0. 0.061) did not significantly affect $\delta^{13}C$ and $\delta^{15}N$ values. We found no significant interactions between the covariates, so interactions were not included in the reporting of these models. There were also no obvious deviations from homoscedasticity or normality upon inspection of residual plots for the final models selected.

From these results, we arrived at the following optimal mixed-effects repeated measures models (Table 2):

$$\hat{\delta N}_{ij} = (\beta_0 + b_0) + (\beta_1 + b_1)Time_{ij} + \beta_2 Time_i^2 + \beta_3 Length_i + \epsilon_{ij}$$

$$\hat{\delta C}_{ij} = (\beta_0 + b_0) + (\beta_1 + b_1)Time_{ij} + \beta_2 Time_i^2 + \beta_3 Length_i + \epsilon_{ij}$$

Where the random effects distributions were $b_0 \sim N(0, \sigma_0^2)$ and $b_1 \sim N(0, \sigma_1^2)$, and $\epsilon_{ij} \sim N(0, \sigma^2)$

2.4.2 Seasonal differences in isotopic niche width

The data show that large seals had a higher variability in whisker $\delta^{13}C$ and $\delta^{15}N$ values, while small seals show less variable whisker $\delta^{13}C$ and $\delta^{15}N$ values. In the summer, the isotopic niche width (SEA_c) was similar for large and small seals, but it became greater for large seals in the fall, winter, and spring (Fig. 3). Dietary overlap among large and small seals was nearly 100% in every season (Fig. 3).

Two small seals (PV09TE016 and PV10TE032), and three large seals (PV10TE010, PV10TE088, and PV10TE094) are notable outliers in their stable isotope values over time. These seals exhibited a marked drop in both $\delta^{13}C$ and $\delta^{15}N$ values in the fall followed by a steep increase in the winter (Fig. 2).

2.5 Discussion

Harbor seals captured in Tracy and Endicott arms during the summer had a seasonal dietary pattern in which whisker $\delta^{13}C$ values increased through the fall, peaked in the winter, and declined through the spring. Whisker $\delta^{15}N$ values showed the opposite trend, peaked in the summer, declined through the fall and winter, and increased again in the following spring (Fig. 2). There was also significant variation among individual seals in both absolute stable isotope ratios and trends through time. Isotopic niche width was greater among large seals across all seasons; however, the difference was greatest during the non-breeding seasons (fall, winter, and spring). The isotopic niche of large seals overlapped that of small seals across all seasons (Fig. 2). Collectively, these results indicate that seals are present in Tracy and Endicott Arms during the summer share a similar diet in the summer, but dietary diversity and differing prey specialization by individuals appears to increase from the fall to the following spring for large seals.

The data in this study do not indicate a specific harbor seal diet, however they do show that large seals, as a group, have a larger proportion of individuals that specialize isotopically, because the higher variability between individuals indicates more individual specialization (Bearhop et al. 2004). For small seals, there was less isotopic variability between individuals for each season, which either indicates a narrow range of prey consumed consistently through each season, or similarities in a more generalist approach resulting in a more consistent isotopic profile in each season (Bearhop et al. 2004). Size-related differences in foraging have been observed in harbor seals (Beck et al. 2007; Bjorkland et al. 2015; Frost et al. 2001; Hastings et al. 2004; Thompson et al. 1998) and specifically for harbor seals that

use floating glacial ice in the summer (Herreman et al. 2009). Large phocids generally dive for longer bouts than smaller phocids (Beck et al. 2007; Lidgard et al. 2005), potentially relating to the higher energetic cost of diving in smaller seals (Frost et al. 2001; Hastings et al. 2004). The ability of larger seals to dive deeper for longer likely allows them to access a wider range of prey (Lance et al. 2012), and to specialize. This difference in diving capability may explain the larger variation in $\delta^{13}C$ and $\delta^{15}N$ values observed in large seals each season, and demonstrate that smaller seals with limited diving capabilities have access to a narrower range of prey (Bjorkland et al. 2015; Frost et al. 2001; Hastings et al. 2004).

Shifts in $\delta^{13}C$ and $\delta^{15}N$ observed along the length of the harbor seal whiskers indicate a shift in seasonal foraging strategies. Higher $\delta^{13}C$ values are representative of benthic prey and lower $\delta^{13}C$ values are representative of more offshore or pelagic prey (Newsome et al. 2010). Also, higher $\delta^{15}N$ values often indicate an increase in trophic level (Newsome et al. 2010). Harbor seals are generally considered to be a nearshore foraging species, remaining close to shore for short shallow dives (Burton and Koch 1999; Frost et al. 2001; Hastings et al. 2004; Womble et al. 2014). Therefore, we propose that the differences in $\delta^{13}C$ values are driven by changes in pelagic versus benthic prey rather than nearshore versus offshore diet. Our findings suggest that the majority of seals in this population begin to shift toward proportionately more pelagic prey from a higher trophic level in the spring, potentially on species like adult salmon (*Oncorhynchus sp.*), Pacific herring (*Clupea pallasii*), and Capelin (*Mallotus villosus*) (Bjorkland et al. 2015; Herreman et al. 2009). In the fall, after pupping and breeding season, our results suggest that seals shift to proportionately more benthic and higher trophic level prey such as rockfish (*Sebastes sp.*), flounder (*Atheresthes stomias*), and sculpin (*Myoxocephalus sp.)* (Bjorkland et al. 2015; Lance et al. 2012).

Five harbor seals in this study, two small and three large, exhibited a seasonal dietary pattern that diverged from most of the other seals, indicated by a substantial shift toward lower values of both $\delta^{13}C$ and $\delta^{15}N$ in the fall (Fig. 3). One possibility is that these seals are targeting species in the spring that

move seasonally into nearshore areas at high densities to spawn in the late summer and fall. For example, sand lance (*Ammodytes hexapterus*) often display a pelagic $\delta^{13}C$ signature and move to shallow water to spawn from August through October (Robards et al. 1999), while other small pelagic species such as capelin (*Mallotus villosus*) and Pacific herring (*Clupea pallasi*) spawn in shallow water in the spring (Arimitsu et al. 2008; Norcross et al. 2001). In both cases, these lipid-rich prey items are only seasonally accessible to harbor seals that forage in nearshore shallow water. Stable isotope ratios of herring, capelin, and sand lance are isotopically similar, with relatively low $\delta^{13}C$ and $\delta^{15}N$ values compared to larger prey species (Bjorkland et al. 2015; Blundell et al. 2002). However, the narrow range of isotope values for these species may explain the seeming uniformity in harbor seal diet from the spring to fall, particularly in the small seals (Fig. 2). Our results cannot distinguish which of these prey our study seals are consuming, but it does suggest that some of the larger seals shift away from prey with nearshore pelagic isotopic signatures in the summer to more diverse prey in the fall and winter. Womble and Gende (2013) reported that some seals in Glacier Bay, Alaska made frequent and extended trips to pelagic regions near an offshore shelf presumably to forage, and that seals use a variety of foraging tactics throughout the year. Like Womble and Gende (2013), our results show that some individual seals deviate in foraging strategy from the rest of the group, particularly the five seals with low $\delta^{13}C$, selecting offshore pelagic prey in the fall.

This study describes the seasonal dietary diversity in a small population of harbor seals that use glacial ice as haulouts during the summer. We found that seal size influenced isotopic niche width, where small seals had a narrow isotopic niche width throughout the year whereas larger seals had a wider isotopic niche, indicating individual specialization on isotopically distinct prey. Dramatic shifts were observed in five harbor seals in the fall, which suggested there is some benthic or more offshore prey source they are selecting. This is a first step in addressing harbor seal diet using sequential isotopic analyses of harbor seal whiskers, and future whisker analysis could be used to help define broader management

concerns, such as: (1) describing dietary differences between adult male and female seals, (2) determine differences in prey consumed between pregnant and non-pregnant females, (3) establish if seasonal diet trends are affected by shifts in ocean conditions, or (4) contrasting prey selection between seals that use terrestrial and glacial haulout sites. Sequential whisker stable isotope analyses are a relatively inexpensive method that holds much promise to address fundamental questions in phocid/harbor seal ecology.

2.6 Acknowledgments

We thank the National Marine Fisheries Service (NMFS) for funding provided through congressional appropriations, the National Fish and Wildlife Foundation (grant #28217) for funding stable isotope analyses, and the Alaska Department of Fish & Game Graduate Award Program for additional support. Additionally, we thank the ADF&G staff and affiliates from 2003-2012 for sample collection and treasured experiences, specifically C. Schmale, G. Blundell, and J. Barton; the Alaska Stable Isotope Facility (T. Howe and N. Haubenstock) for their direction and sample analysis; We are grateful to G. Breed and P. Barboza for their guidance; and D. Foley of the M/V *Steller*.

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Table 2.1. Harbor seal morphometric data collected during 2009 and 2010 in Tracy Arm Fords Terror, Alaska .

Seal ID	Sex	Capture Date	Curvilinear Length (m)	Axial Girth (m)	Weight (kg)
PV09TE014	Male	5/4/2009	1.39	1.00	53.00
PV09TE025	Male	5/5/2009	1.35	0.94	46.30
PV09TE034	Male	5/7/2009	1.34	0.95	49.50
PV09TE041	Male	5/8/2009	1.37	0.86	46.90
PV09TE044	Male	5/8/2009	1.35	0.99	53.00
PV09TE046	Male	5/8/2009	1.42	1.00	59.80
PV09TE063	Male	5/10/2009	1.33	1.01	52.30
PV09TE086	Male	7/5/2009	1.56	1.18	92.20
PV10TE003	Male	4/29/2010	1.66	1.26	110.00
PV10TE010	Male	4/30/2010	1.49	0.88	64.80
PV10TE032	Male	5/2/2010	1.37	0.99	54.60
PV10TE037	Male	5/3/2010	1.35	0.93	51.80
PV10TE065	Male	5/6/2010	1.48	1.05	65.40
PV09TE058	Female	5/10/2009	1.36	0.92	43.00
PV09TE040	Female	5/7/2009	1.43	1.00	56.00
PV09TE018	Female	5/5/2009	1.32	0.90	45.80
PV09TE016	Female	5/5/2009	1.36	0.95	52.00
PV09TE011	Female	5/4/2009	1.30	1.04	55.00
PV09TE083	Female	7/4/2009	1.52	0.93	52.40
PV09TE105	Female	7/8/2009	1.46	0.93	49.70
PV09TE107	Female	7/8/2009	1.56	1.00	59.10
PV09TE159	Female	7/13/2009	1.25	0.94	43.60
PV10TE001	Female	4/29/2010	1.27	0.98	48.90
PV10TE013	Female	4/30/2010	1.32	0.92	46.80
PV10TE014	Female	4/30/2010	1.23	1.02	49.60
PV10TE015	Female	4/30/2010	1.27	0.97	47.60
PV10TE016	Female	4/30/2010	1.60	1.02	71.40
PV10TE038	Female	5/4/2010	1.31	0.97	48.80
PV10TE054	Female	5/5/2010	1.38	0.94	53.40
PV10TE088	Female	7/12/2010	1.44	1.01	59.30
PV10TE094	Female	7/13/2010	1.44	0.97	59.10
PV10TE132	Female	7/14/2010	1.50	1.13	81.40

Table 2.2. List of candidate models describing the response of $\delta^{13}C$ and $\delta^{15}N$ values in harbor seal whiskers using corrected Akaike Information Criterion (AICc) and log-likelihood ($\log(\mathcal{L})$) values. Models are ranked by AICc values from lowest to highest. Fixed effects include T = Time, T^2 = Time^2, S = Sex, Y = Year of capture, and C = Curvilinear length.

Isotope	Rank	Model	AICc	$\log(\mathcal{L})$
$\delta^{15}\widehat{N}$	1	$T+T^2+S+C+(1+T\|ID)$	597.2	-289.6
	2	$T+T^2+S+Y+C+(1+T\|ID)$	597.8	-288.9
	3	$T+T^2+C+(1+T\|ID)$	598.7	-291.4
	4	$T^2+C+(1+T\|ID)$	619.2	-302.6
	5	$T+T^2+C+(1\|ID)$	628.4	-309.2
	6	$T+C+(1+T\|ID)$	628.5	-307.3
	7	$T+T^2+S+Y+C+(1+T\|ID)+(1+T^2\|ID)$	815.5	-394.2
$\delta^{13}\hat{C}$	1	$T+T^2+C+(1+T\|ID)$	527.7	-255.9
	2	$T+T^2+S+C+(1+T\|ID)$	529.7	-255.9
	3	$T+T^2+S+Y+C+(1+T\|ID)$	531.7	-255.9
	4	$T+T^2+(1+T\|ID)$	531.7	-258.8
	5	$T^2+C+(1+T\|ID)$	577.0	-281.5
	6	$T+C+(1+T\|ID)$	578.0	-282.0
	7	$T+T^2+S+C+(1\|ID)$	582.0	-284.0
	8	$T+T^2+S+Y+C+(1+T\|ID)+(1+T^2\|ID)$	669.7	-321.9

Table 2.3. Mean (± 1 SE) stable carbon and nitrogen isotope ratios from whisker sections of 32 different harbor seals. Seals were grouped by curvilinear length (m). Serial sections along the whisker were translated into calendar date, and mean stable carbon and nitrogen isotope ratios were calculated for the summer = 4/29/09 – 7/20/09, fall = 7/21/09 – 10/28/09, winter = 10/29/09 – 2/5/10, and spring = 2/6/10 – 5/16/10.

Seal Size	Season	*n*	$\delta^{13}C$	$\delta^{15}N$
Large				
(≥ 1.4 m)	Summer	47	-14.26 ± 0.09	16.01 ± 0.17
	Fall	74	-14.48 ± 0.22	15.87 ± 0.28
	Winter	35	-13.87 ± 0.15	16.04 ± 0.16
	Spring	21	-14.10 ± 0.19	16.11 ± 0.18
Small				
(< 1.4 m)	Summer	111	-14.53 ± 0.07	15.53 ± 0.09
	Fall	61	-14.48 ± 0.10	15.32 ± 0.10
	Winter	55	-14.95 ± 0.06	15.48 ± 0.10
	Spring	20	-14.94 ± 0.08	15.97 ± 0.11

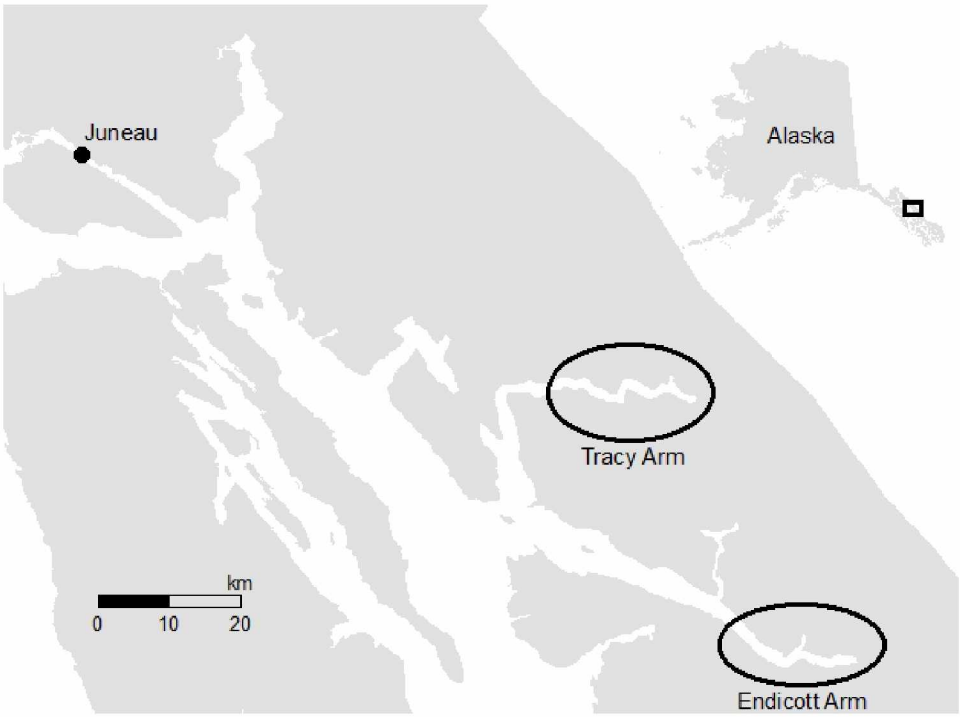

Figure 2.1. Harbor seals captured in the glacial fjores in southeast Alaska at the ends of Tracy and Endicott Arms, in the Tracy Arm Fords Terror Wilderness Area, Alaska.

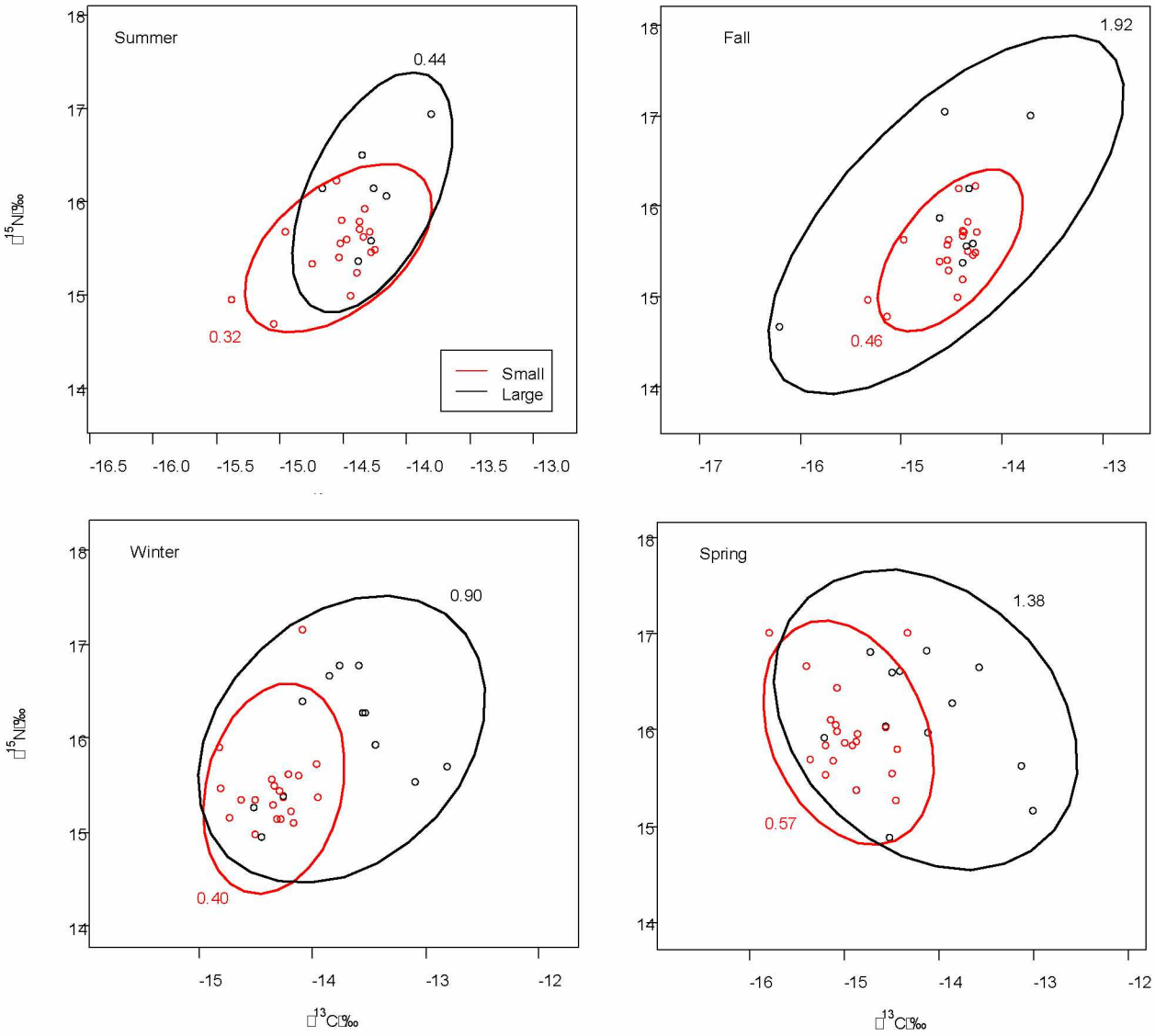

Figure 2.2 Harbor seal niche width are represented by standard ellipse area (SEA_c ‰^{2}), with SEA_c values presented next to drawn ellipses. Seals were grouped by curvilinear length (m); large ≥ 1.4 m (black), and small < 1.4 m (red). Serial sections along the whisker where translated into calendar dates and values of $\delta^{13}C$ and $\delta^{15}N$ were averaged within the time periods for each whisker; summer = 4/30/09 – 7/20/09, fall = 7/21/09 – 10/28/09, winter = 10/29/09 – 2/5/10, spring = 2/6/10 – 5/16/10, and the following summer = 5/17/10 – 7/14/10. See Table 4 for number of samples per mean and standard error around the mean $\delta^{13}C$ and $\delta^{15}N$ values.

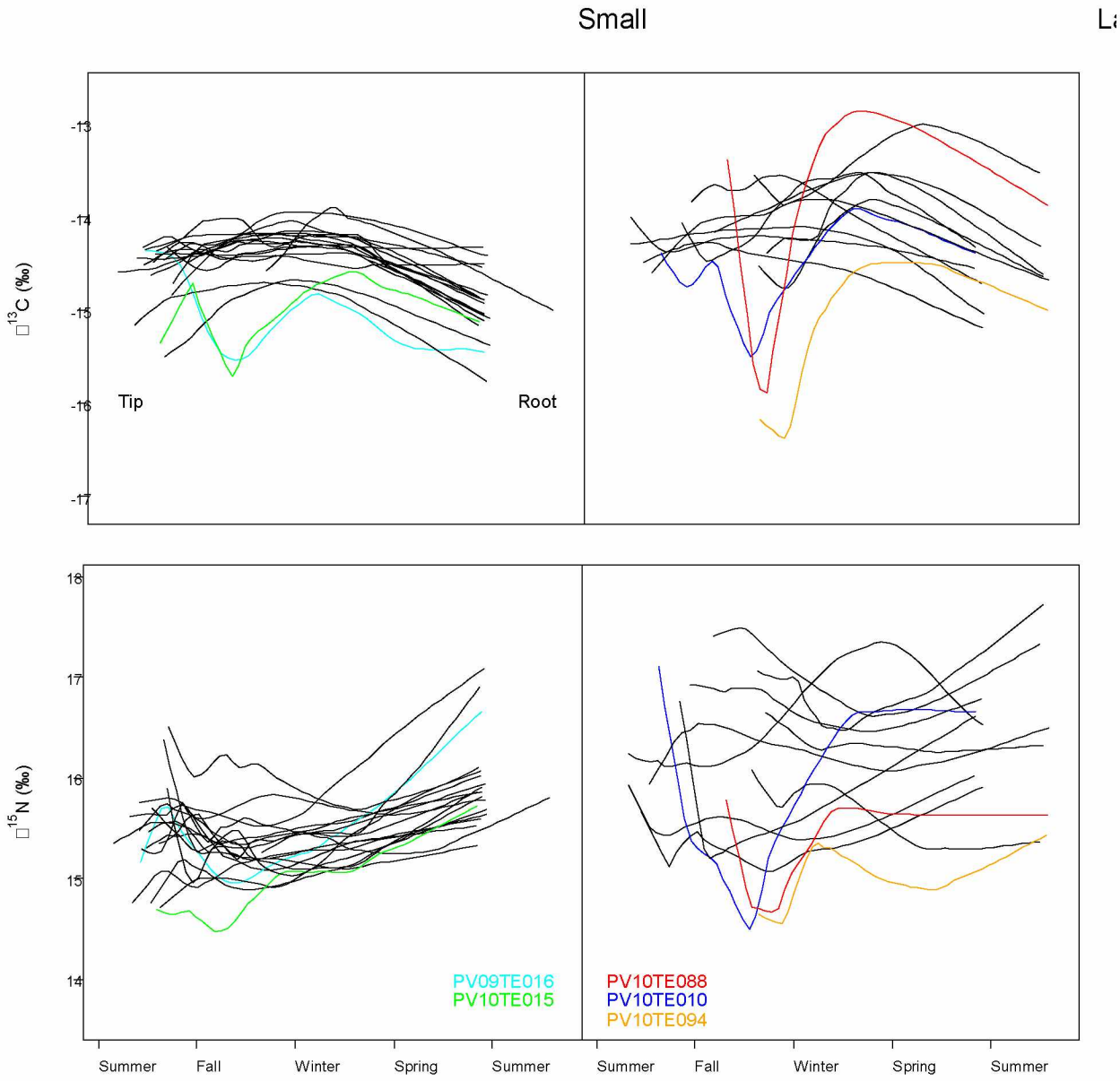

Figure 2.3 $\delta^{13}C$ and $\delta^{15}N$ values in harbor seal whiskers (Row) plotted over time by group (Column) Seals were grouped by curvilinear length (m); large (≥ 1.4 m), and small (< 1.4 m). Time periods on the x-axis are defined as: summer = 4/30/09 – 7/20/09, fall = 7/21/09 – 10/28/09, winter = 10/29/09 – 2/5/10, spring = 2/6/10 – 5/16/10, and the following summer = 5/17/10 – 7/14/10. Five seals that displayed deviations in the fall are highlighted in color for comparison. Stable isotope ratios were smoothed using a smoothing function in the Lattice package for R. See Table 2 for number of samples per mean and standard error around the mean $\delta^{13}C$ and $\delta^{15}N$ values.

Conclusion

Harbor seal (*Phoca vitulina*) populations in the Gulf of Alaska that use tidewater glacier habitats in the summer to molt, pup, and breed have sharply declined in recent years (Hoover-Miller et al. 2011; Mathews and Pendleton 2006; Womble et al. 2010). One possible explanation for the decline is shifts in prey quality or availability due to marine ecosystem changes (Anderson et al. 1999; Hirons et al. 2001). While previous work described the diet of harbor seals using glacial areas during the summer in the Gulf of Alaska (Herreman et al. 2009; Womble et al. 2014), diet remains understudied for these seals over the rest of the year. Measuring stable carbon and nitrogen isotope ratios in serial sections of whiskers is a way to estimate a temporal record of diet for up to one year. However, the variability in individual whisker growth and replacement has been a barrier to estimating when stable isotopes are deposited into the whisker (Beltran et al. 2015; Greaves et al. 2005; Hirons et al. 2001; Lübcker et al. 2016; Zhao and Schell 2004). In the first chapter of this thesis I evaluated whether whisker morphological features can be used to improve estimates of the timing of stable isotope deposition into harbor seal whiskers. I proposed adjustments to the von Bertalanffy (1968) growth model to incorporate individual variations in whisker growth rates when transforming whisker lengths into deposition date estimations. I then used these morphological features of harbor seal whiskers to choose whiskers with similar growth rates and shedding date estimates to describe the seasonal trends in stable isotopes for two size classes of harbor seals in the Gulf of Alaska that use tidewater glaciers to haul out in the summer. The results presented in this thesis suggest that whisker morphometric characteristics of harbor seals can be used to improve the ability for managers to make inferences using chemical markers in serial sections of whiskers.

In Chapter 1, I characterized harbor seal whisker morphology and tested different approaches to estimating deposition date. I found (1) that a prominent smooth root section (SRS) can be used to identify whiskers that are close to fully grown, (2) the SRS can be used to approximate when whiskers of different age and sex classes are shed, and (3) the inter-bump length (IBL) can be used as a metric to

define whisker growth rates as a function of whisker length. The described correlation between total whisker length and the length of the SRS allows researchers to estimate when the whisker began growing. This will help with the selection of whiskers that are most similar in growth status when examining stable isotopic signatures to reconstruct a seasonal dietary profile. By combining previously described growth rates and the findings in Chapter 1, harbor seal whisker morphometric characteristics can be used to improve estimates of when stable isotopes, or other chemical markers, were deposited along the length of the whisker.

In Chapter 2, I described longitudinal isotopic profiles of a population of harbor seals that molt, pup, and breed in glacial-fed fjords in southeast Alaska. I found both large and small seals shared a similar isotopic niche width in the summer, while large seals, as a group, had a larger niche width in the fall, winter, and spring. This high individual variation spanning large isotopic space indicates individual specialization on distinct prey outside of the pupping and breeding season. One unexpected finding was five seals (two small, three large) that showed a sharp decrease in $\delta^{13}C$ and $\delta^{15}N$ values after the pupping and breeding season. This pattern deviates from the more moderate shift to lower $\delta^{13}C$ and higher $\delta^{15}N$ values during that same period exhibited by the rest of the population. These results were similar to Womble and Gende (2013), who found that some harbor seals travel offshore to the continental shelf, presumably to forage on pelagic fishes, unlike most harbor seals that remain nearshore and forage in the benthos. The results in Chapter 2 are consistent with past research describing the isotopic niche width of harbor seals that use tidewater glaciers in the summer, where seals generally forage on similar prey items in the summer and shift foraging patterns in the fall, while larger seals show a wider variability in what they eat from fall to the following spring (Bjorkland et al. 2015; Herreman et al. 2009). However, this study is unique in using whiskers to record longitudinal markers as a representation of diet for a population of harbor seals in all seasons and for two different size classes.

The findings in this thesis suggest several promising directions for future research. In addition to estimating the seasonal diet as it relates to harbor seal size, we can expand our scope of inference with increased whisker sampling. Broad-scale sampling could improve our understanding of niche widths of all demographic groups on a geographic or temporal scale. For example, our data suggest that the majority of seals that use tidewater glacial habitat are targeting prey species in the spring that move seasonally into nearshore areas at high densities to spawn in shallow water (i.e. Pacific herring; *Clupea pallasi*) (Arimitsu et al. 2008; Norcross et al. 2001). Steller sea lions (*Eumetopias jubatus*), commercial fishermen, and other marine predators also utilize Pacific herring at this time of the year (Womble et al. 2005). Future researchers may now use stable isotopes sampled from archived whiskers to ask which seals are targeting this prey in the spring. Further, questions can be answered regarding a change in stable isotope patterns in harbors seals that haul out on terrestrial sites, and if these prey changes are related to population declines. Continually growing keratinous whiskers are useful beyond estimating diet through stable isotopes. Longitudinal data from keratinous structures can also be used to describe contaminants (Rea et al. 2013) and steroid hormones. The use of phocid whiskers to describe temporal trends in seal life history continues to be explored, and the efforts here are the first step of assessing chemical markers in serial sections of the whisker.

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